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(71)(72) Applicants and Inventors: NG, Gordon, Y., K. [CA/CA]; 38 Elm Street #3300, Toronto, Ontario M5G 2K5 (CA). SEEMAN, Philip [CA/CA]; 32 Parkwood Avenue, Toronto, Ontario M4V 2X1 (CA). GEORGE, Susan, R. [CA/CA]; 54 Thombank Road, Thornhill, Ontario K4J 2A4 (CA). O'DOWD, Brian, F. [CA/CA]; 229 Catalina Drive, Scarborough, Ontario M1A 1B8 (CA).

(74) Agent: RAE, Patricia, A.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

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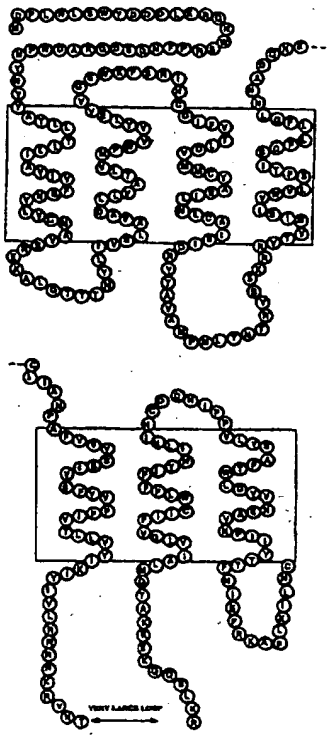
(54) Title: RECEPTOR AND TRANSPORTER ANTAGONISTS

(57) Abstract

Specific antagonists for prokaryotic or eukaryotic integral membrane proteins are provided. The antagonists are peptides having the amino acid sequence of a transmembrane domain of the integral membrane proteins or of a portion of analogue thereof. Methods are provided for preventing or treating disorders characterised by disordered function of an integral membrane protein by administration of a specific peptide antagonist of the integral membrane protein.



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RECEPTOR AND TRANSPORTER ANTAGONISTS

Related Applications:

This application claims priority from U.S. Provisional Patent Application No. 60/014,306 filed on March 27, 1996, U.S. Patent Application No. 08/670,119, filed June 25, 1996 and U.S. Provisional Patent Application No. 60/024,240 filed August 20, 1996.

Field of the Invention

This invention relates to the field of integral membrane proteins which act as receptors or signal transducers. More specifically, it relates to the identification and preparation of specific antagonists of the function of such proteins.

Background of the Invention

Various journal articles referred to herein are identified by authors and date in parentheses and are listed, with full citations, at the end of the specification.

Receptors are the primary targets and mediators of hormone and drug actions. The cell surface receptors, such as the G protein-coupled receptors (GPCRs), ion channel receptors, immunoglobulin receptors and tyrosine kinase receptors, belong to gene superfamilies based on sequence and structural similarities. Receptors belonging to these superfamilies are all integral membrane proteins predicted to exhibit extracellular, hydrophobic membrane-spanning and intracellular domains. Whereas tyrosine kinase receptors and most immunoglobulin receptors exhibit a single membrane spanning domain, G protein-coupled receptors are defined by seven putative hydrophobic membrane spanning segments which have become

the hallmark of this gene superfamily. The biogenic amine transporter proteins are also membrane-spanning proteins, with twelve transmembrane segments, that mediate the reuptake of released neurotransmitter. The ion channel receptors generally have separate subunits that associate together to form a functional receptor.

The amino acid sequence of a receptor protein, which is unique to each receptor, confers specific structure-related functions to the receptor, while conforming to the general structural determinants of the particular class of protein to which it belongs. The amino acid sequence of an integral membrane protein, such as a receptor or transporter protein, determines the hydrophobic and hydrophilic portions, and has been used in the development of algorithms for the prediction of membrane protein secondary structure (Engelman et al., 1986, Kyte and Doolittle, 1982). Peptide probes derived from regions of various proteins have been used for mapping structural determinants of proteins. This has been best characterized for the single transmembrane spanning sialoglycoprotein, glycophorin A, isolated from human erythrocyte membranes (Furthmayr and Marchesi, 1976). In these studies, a small hydrophobic peptide derived from glycophorin A was able to prevent the association of 2 subunits *in vitro*, which suggested that native glycophorin A in membranes is composed of subunits associated with each other by hydrophobic portions of the polypeptide chains. Subsequent studies demonstrated that a specific molecular motif (LIxxGVxxGVxxT) participates in the formation of glycophorin A dimers (Bormann et al., 1989, Lemmon et al., 1992).

For the G protein coupled receptors, Okamoto et al. (1991) used a synthetic peptide corresponding to the end of the third cytoplasmic loop of the G protein-coupled

β2-adrenergic receptor to demonstrate that this amino acid sequence was critical for this receptor's ability to activate G proteins. In addition, it has been shown that coexpression of the third intracellular domain of the G protein-coupled α1B-adrenergic receptor, along with the receptor specifically inhibited receptor-mediated inositol phosphate production (Luttrell et al., 1993), and that expression of the intracellular third loop of the dopamine D1 receptor specifically inhibited receptor-mediated cAMP production (Hawes et al., 1994).

For the tyrosine kinase receptors, the investigation of an activating mutation of the *neu* oncogene in the rat revealed that co-expression of short transmembrane molecules encoded by DNA constructs retarded the growth of *neu* transformed cells (Lofts et al., 1993).

GPCRs have been shown to exist as dimers and monomers in the presence of denaturants and reducing agents (Ng et al., 1993, 1994a, 1994b), suggesting that dimerisation does not occur via covalent disulphide bonds. It has been hypothesised that the seven transmembrane(TM) spanning domains of GPCRs are sequentially arranged in an anti-clockwise circular manner forming a TM receptor core (Baldwin, 1993). The proper folding of GPCR monomers is suggested to involve intramolecular interactions between TMI and TMVII (Kobilka et al., 1988, Suryanarayana et al., 1992). Coexpression studies with chimeric GPCRs have suggested that receptor interactions may involve TMVI and VII (Maggio et al., 1993, Liu et al., 1995), and mutagenesis studies have demonstrated that antagonist interaction with the receptors may involve these TM regions.

The importance of membrane-spanning proteins such as receptors and transporters in signal transmission across the cell membrane makes these proteins prime targets for

pharmaceutical intervention in many disorders. There are presently few highly selective pharmaceuticals available for treatment of such disorders. There is therefore a great need for drugs useful for the prevention or treatment of receptor-mediated disorders, and for the selective targeting of receptor/transporter functions, to aid in the treatment of commonly occurring and highly prevalent diseases.

Brief Description of the Drawings

The invention, as exemplified by preferred embodiments, is described with reference to the accompanying drawings in which:

Figure 1 shows a two dimensional representation of the seven membrane-spanning domains of the D2 dopamine receptor.

Figure 2 shows a two dimensional representation of the single membrane-spanning domain of the epidermal growth factor receptor.

Figures 3A to 3G show immunoblots of isolated dopamine receptors after various treatments, monomers (M) and dimers (D) being indicated by arrows.

Figure 3A shows the effect of the indicated concentrations of D2-TM VI peptide (aa 375-394) on the electrophoretic pattern of the D2 receptor (Lanes 1-4: 0, 1.3, 1.6 and 2.5 mg/ml D2-TM VI peptide, respectively).

Figure 3B shows the effect of the indicated concentrations of D2-TM VII peptide (aa407-426) on the D2 receptor (Lanes 1-7: 0, 0.3, 0.6, 1.0, 1.3, 1.6 and 2.5 mg/ml D2-TM VII peptide).

Figure 3C shows the effect of incubation with the D2-TM VI and D2-TM VII peptides on the D2 receptor from human caudate nucleus. Lanes 1 and 3: buffer control;

lane 2: D2-TM VI peptide; lane 4: D2-TM VII peptide.

Figure 3D shows the effect of hydrophilic and hydrophobic receptor peptides on a D2 receptor preparation. D2 receptors were incubated in peptide buffer (lane 1); D2-C IIIA peptide (aa 244-263) (lane 2); D2-C IIIB peptide (aa 284-303) (lane 3); β2-AR TM VI peptide (aa 276-296) (lane 4); D1-C IIIA peptide (aa 369-383) (lane 5); and D1-C IIIB peptide (aa 416-431) (lane 6).

Figure 3E shows the effect of D2-TM VII peptide on c-myc epitope-tagged human dopamine D1 receptor and c-myc epitope-tagged human serotonin 5-HT1B receptor. D1 receptors were incubated in peptide buffer without (lane 1) or with (lane 2) D2-TM VII peptide, and 5-HT1B receptors were incubated in peptide buffer without (lane 3) or with (lane 4) D2-TM VII peptide.

Figure 3F shows the effect of temperature on the D2 dopamine receptor.

Figure 3G shows the effect of pH on the D2 dopamine receptor (GAA = glacial acetic acid).

Figure 4 shows the dose-dependent inhibition of [^{3}H]spiperone binding to membranes prepared from D2 receptor-expressing Sf9 cells by various receptor subtype-specific peptides. The peptides tested were: D2-TM 7 (TWLGYVNSA) (▲), D2-TM 5 (PAFVVYSSIVSFYVPFIVTL) (Δ), GABA-TM (GIFNLVYW) (□), V2-TM7 (LMLLASLNSCTNPWIY) (■) and DAT-TM12 (ALGWIIATS) (o). The effect of various concentrations of peptides is shown as an average percentage of the total [^{3}H]spiperone binding from 2 or more independent experiments.

Figure 5 shows the dose-dependent competition displacement of agonist [^{3}H]quinpirole binding (shown as percentage of total [^{3}H] quinpirole binding) to membranes prepared from D2 receptor-expressing Sf9 cells by D2

receptor antagonist spiperone (o) and D2-TM 7 peptide, TWLGYVNSA (•).

Figure 6 shows D2 receptor mediated dose-dependent dopamine activation of [^{35}S]GTPγS binding (left panel), and the dose-dependent inhibition of dopamine activation of [^{35}S]GTPγS binding by the D2 antagonists, spiperone (shaded bar) and D2-TM 7 peptide (TWLGYVNSA) (open bar) (right panel). D2 receptor mediated dopamine activation of [^{35}S]GTPγS binding (left panel) is shown as a percent of baseline activity, and the inhibition of dopamine activation of [^{35}S]GTPγS binding by the D2 antagonists, spiperone and D2-TM 7 peptide (TWLGYVNSA) (right panel) is shown as a percentage of the maximal response.

Figure 7 shows D2 receptor mediated dose-dependent dopamine activation of [^{35}S]GTPγS binding (left panel), and the dose-dependent inhibition of dopamine activation of [^{35}S]GTPγS binding by the D2-TM 7 peptide (TWLGYVNSA) (open bar) and the GABA-TM peptide (GIFNLVYW) (solid bar) (right panel). D2 receptor mediated dopamine activation of [^{35}S]GTPγS binding (left panel) is shown as a percent of baseline activity, and the inhibition of dopamine activation of [^{35}S]GTPγS binding by the D2-TM 7 peptide and GABA-TM peptide (right panel) is shown as a percentage of the maximal response. The values shown are the average from 2 or more independent experiments.

Figure 8 shows the effect of co-expression of a D2-TM7 peptide and the full length D2 receptor on D2 receptor density in COS cells, estimated by spiperone binding. Co-expression of a D2-TM7 peptide and the D2 receptor (o) was compared with expression of the full length D2 receptor alone (•). A representative of two independent experiments is shown.

Figure 9 shows the effect of coexpression of a D2-TM7 peptide with full length D2 receptors on D2 receptor function in COS cells (shaded bar) compared with expression of the full length D2 receptor alone.

Figures 10A to 10C show the duration (X axis) and extent (Y axis) of asymmetric body response of a rat after unilateral (left) intrastriatal injection of D2-TM VII peptide (LYSAFTWLGYVNSAVNPIIY), 15 ng/3 µl (Fig. 10A), peptide vehicle (Fig. 10B), and β2-AR VI peptide (GIIMGTFTLCWLPFFIVNIVH-COOH), 15 ng/3 µl (Fig. 10C).

Figure 11 shows the duration (X axis) and extent (Y axis) of asymmetric body response of a rat with bilateral cannulae after left intrastriatal injection of D2-TM VII peptide (TWLGYVNSA), 15 ng/3 µl (together with vehicle injection into the right striatum concurrently).

Figure 12 shows immunoblots of an Ni-NTA resin-purified preparation from Sf9 cells co-expressing a c-myc epitope-tagged, β2-adrenergic receptor (-c-myc-β2AR+) and a histidine-tagged TMVII peptide of the β2-adrenergic receptor (6xHis-β2AR-TMVII) probed (left panel) with monoclonal antibody 9E10 against the c-myc epitope and (right panel) with a polyclonal antibody against the poly-histidine sequence of the TMVII β2-adrenergic receptor peptide. Left lane of each panel shows molecular mass standards.

Figure 13A shows the dose-dependent inhibition of [^{3}H]alprenolol binding to membranes prepared from β2-adrenergic receptor-expressing Sf9 cells by various receptor subtype-specific peptides. The peptides tested were: β2AR-TM I (LLIVVGNVLVI), β1AR-TMVII (GYANSAFNP), CCR5-TMI (LYSLVFIFGFVGN), Fo c-TM (*E. Coli* ATPase Fo c subunit: GQAIAFVLFVL) and Fo b-TM (*E. Coli* ATPase Fo b

subunit: LAAIGAAIGIGILG). Figure 13B shows the effect of the prototypical adrenergic receptor antagonist pindolol for comparison. Inhibition is represented as the percentage of the total [^{3}H]alprenolol binding.

Figures 14A to 14H show polygraph traces of blood pressure (Y axis: mm Hg) in a rat at indicated time intervals (X axis) after treatment with various agents:

Figure 14A: baseline;

Figure 14B: 1 mg/Kg isoproterenol (β1-AR agonist);

Figure 14C: 500 mg LYSAFTWLGYUNSAVNPIIY-NH2 (β1-AR TMVII peptide); Figure 14D: 1 mg/Kg isoproterenol;

Figure 14E: baseline;

Figure14F:1mg/Kgisoproterenol; Figure 14G: vehicle;

Figure 14H: 1 mg/Kg isoproterenol.

Figures 15A to 15K show polygraph traces of blood pressure (Y axis: mm Hg) in a rat at indicated time intervals (X axis) after treatment with various agents:

Figure 15A: baseline;

Figure 15B: 5 mg/Kg phenylephrine (α1A-AR agonist);

Figure 15C: 500 mg FFNWLGYANSAFNP-NH2 (α1A-AR TM7 peptide); Figure 15D: 5 mg/Kg phenylephrine; Figure 15E: saline; Figure 15F: 5 mg/Kg phenylephrine; Figure 15G: vehicle; Figure 15H: baseline; Figure 15I: 5 mg/Kg phenylephrine; Figure 15J: 1 mg/Kg prasozin (α1A-AR antagonist); Figure 15K: 5 mg/Kg phenylephrine.

Figure 16 shows urine output (Y axis) as a function of time (X axis) in a representative unilaterally nephrectomized rat under anesthesia after treatment with vehicle (10% DMSO) in water) or V2 receptor TM VII antagonist peptide, LMLLASLNSCTNPWIY, (V2 AT).

Figures 17A and 17B show the effect of a CCR5-TM I peptide antagonist, (CCR5-TMI: LYSLVFIFGFVGN) on HIV infection of PBMC cells as assessed by HIV reverse

transcriptase activity (Figure 17A) and by HIV P24 antigen production (Figure 17B).

Figure 18 shows dose-dependent inhibition of EGF receptor tyrosine kinase activity in solubilized membrane preparations from cultured A431 cells by various transmembrane peptides. LTVIAGLVVIF, a peptide derived from the TM domain of the EGF-erb3 receptor (EGF-tm: solid bars) was compared with a peptide derived from the TM domain of the GABA-A receptor subunit, GIFNLVYW (GABA-tm: open bars). Response is shown as percentage of total EGF receptor-mediated, $^{32}P\gamma$ incorporation into a receptor-specific substrate.

Figure 19 shows the antimicrobial effect of various transmembrane-based peptides on *E. Coli* as assessed by plating and counting growth of colonies on Luria Broth plates. Peptides examined were GIFNLVYW (GABA-TM), LAAIGAAIGIGILG (Fo c-TM), and GQAIAFVLFVL (Fo b-TM).

Figures 20 to 22 show the effect of intra-cerebral (IC) injection of the peptide ALGWIIATS on dopamine release (X axis) at various time intervals (Y axis) in caudate nucleus and *nucleus accumbens* measured by *in vivo* microdialysis in an awake rat.

Figure 20 shows the release of dopamine in striatum as a function of time following cocaine administration (5 mg/Kg) with no pre-treatment (•) or with pre-treatment of rats with IC injection of DAT-TM 12 peptide (ALGWIIATS) 15 min before cocaine administration (o). Dopamine release is shown as a percentage of basal values.

Figure 21 shows the effect of IC injection of the DAT-TM 12 peptide, ALGWIIATS, alone. Response is shown as percentage basal striatal release as a function of time.

Figure 22 shows the release of dopamine in the *nucleus accumbens* as a function of time following cocaine administration (5 mg/Kg) with no pre-treatment

(•) or with pre-treatment of rats with IC injection of DAT-TM 12 peptide, ALGWIIATS, 5 min before cocaine administration (o). Dopamine release is reported as a percentage of basal values.

Figure 23 shows the effect of a CD4-TM peptide antagonist, LIVLGGVAGLLLF, on HIV infection of PBMC cells as assessed by HIV P24-antigen production.

Detailed Description of the Invention

The inventors have shown that a peptide which has the amino acid sequence of a hydrophobic or transmembrane (TM) domain of an integral membrane protein, or of a portion of a transmembrane domain, has a specific and selective antagonistic effect on the activity or function of the integral membrane protein from which it is derived. This antagonistic effect has been shown both *in vitro* and *in vivo*, in animal models.

While the methods and antagonist peptides of the invention may be utilised in advance of a complete understanding of their mechanism of action, it is hypothesised that the mechanism of the antagonism exerted by transmembrane domain peptides is the binding of such peptides to a transmembrane domain of the integral membrane protein, thereby interfering with intramolecular interactions which contribute to the proper three-dimensional conformation of the integral membrane protein monomer. It is hypothesised that the formation of a heterodimer of the antagonist peptide and the integral membrane protein monomer will interfere with binding of the integral membrane protein with its ligand and, for integral membrane proteins which are normally associated as dimers, will interfere with dimer formation.

It is predicted that all integral membrane proteins having one or more transmembrane domains will be

susceptible to disruption of their function by a peptide having the amino acid sequence of any one of their own transmembrane domains. An antagonist peptide having a transmembrane amino acid sequence of a particular integral membrane protein shows specificity for that protein and does not interfere with the function of closely related integral membrane proteins.

Integral membrane proteins comprise a great variety of proteins, including signal-transducing proteins such as G-protein coupled receptors and tyrosine kinase receptors, transporter proteins, membrane channel proteins, immunoglobuin receptors and adhesins.

Integral membrane proteins are found in the cell membranes of prokaryotic and eukaryotic cells and also within intracellular membranes in eukaryotic cells, for example the endoplasmic reticulum intracellular transporter, lysosomal membrane proteins and sialoglycoproteins. Antagonist peptides in accordance with the present invention can be used to control the function of integral membrane proteins found in all these various locations.

Also included within the scope of the invention are fragments or analogues of the transmembrane amino acid sequences of an integral membrane protein which are effective to antagonise the function of that protein. A fragment or analogue of a transmembrane amino acid sequence of an integral membrane protein is effective if it is a functional equivalent of the transmembrane amino acid sequence.

The transmembrane or membrane-spanning domains of integral membrane proteins are believed to have a helical conformation and generally comprise a sequence of about 22 to 26 amino acids. In some integral membrane proteins, the transmembrane domains adopt a barrel

conformation.

The antagonist peptide for a particular integral membrane protein may have the entire amino acid sequence of a transmembrane domain or may comprise a portion or fragment of the transmembrane amino acid sequence.

Fragments of a transmembrane amino acid sequence may be selected by truncation of one or more amino acids from the amino terminus of the transmembrane amino acid sequence, by truncation of one or more amino acids from the carboxy terminus or by truncation of one or more amino acids from both amino and carboxy termini.

As is understood by those skilled in the art, in the identification of a transmembrane amino acid sequence within the total amino acid sequence of an integral membrane protein, there may be a variation of one or two amino acids in defining the termini of the transmembrane amino acid sequence, depending on the hydropathy analysis software used.

This possible variation does not limit the ability of one skilled in the art to select antagonist peptides or fragments in accordance with the invention.

The present invention provides antagonist peptides which correspond to the amino acid sequence of an integral membrane protein transmembrane domain, fragments of such a TM amino acid sequence and peptides which include the amino acid sequence of an integral membrane protein transmembrane domain or fragments thereof.

The present invention provides antagonist peptides comprising amino acid sequences corresponding to at least four, preferably ten and more preferably from fifteen to twenty consecutive amino acids of an integral membrane protein transmembrane domain.

The amino acid sequences of the transmembrane domains of integral membrane proteins are highly

conserved in mammals.

The function of an integral membrane protein from a first species may be antagonised by a peptide corresponding to the amino acid sequence of one of its own transmembrane domains or may be antagonised by a functionally equivalent transmembrane domain amino acid sequence from the corresponding region of the integral membrane protein of a second species. The term "functionally equivalent" means that the sequence of the transmembrane domain of the second species need not be identical to that of the first species but need only comprise a sequence which functions biologically and/or chemically as the equivalent of the transmembrane amino acid sequence of the first species.

The present invention provides a generally applicable means of selecting a suitable specific antagonist for inhibition or reduction of the activity of a target integral membrane protein such as a receptor or transporter.

In addition to the antagonist peptides disclosed herein, one of ordinary skill in the art is enabled by the present invention to identify and prepare antagonist peptides specific for any selected integral membrane protein.

Many databases are available which contain the amino acid sequences of a large number of integral membrane proteins and some databases such as SwissProt database also list the TM domains of these proteins. For proteins whose TM domains are not listed in a database, the amino acid sequence can be subjected to hydropathic analysis, for example using a computer program such as STRIDER, to deduce the TM domain amino acid sequences.

A number of examples of antagonist peptides derived from integral membrane protein TM domains in accordance

with the invention are described herein in detail. Tables 1A to 1D show examples of the G-protein coupled receptors whose amino acid sequences can be accessed in public databases and Table 3 shows examples of other sequences available in databases.

Guided by the present disclosure and using integral membrane protein amino acid sequence information available either in the scientific literature or in databases such as GenBank or SwissProt, one of ordinary skill in the art can examine the hydrophobic transmembrane amino acid sequence or sequences of a selected integral membrane protein and identify a suitable amino acid sequence for an antagonist peptide specific for that protein.

The present invention also enables the rational design of specific antagonist peptides or blockers active against the protein product of any gene predicted to encode an integral membrane protein.

Once the amino acid sequence of a new integral membrane protein is determined, for example by cloning and sequencing a gene or cDNA for the protein and deducing therefrom the amino acid sequence, the amino acid sequence can be subjected to hydropathic analysis, as described above, to identify the TM domains. The amino acid sequence of at least one transmembrane domain is then synthesised to provide a selective peptide antagonist of the integral membrane protein.

Alternatively, suitable effective fragments or analogues of a transmembrane amino acid sequence may be selected and screened as described herein.

The present invention enables novel specific pharmaceuticals for treatment of many disorders. Selection of specific antagonist peptides in accordance with the invention enables the development of

discriminating drugs with previously unavailable selectivity and hence reduced side-effects.

For example, receptor and transporter antagonists may be used to treat disorders associated with specific receptor overactivity such as schizophrenia which is associated with overactivity of the D2 dopamine receptor, or may be used to indirectly restore homeostasis in disorders which do not directly involve aberrant function of the particular receptor or transporter. Examples of disorders and antagonists in the latter category include: anti-D1 dopamine receptor for drug abuse, anti-histamine receptor for peptic ulcer disease, anti-angiotensin receptor for hypertension and anti-β adrenergic receptor for glaucoma.

Using the D2 dopamine receptor as a model for other membrane spanning receptors, the inventors have shown a dopamine antagonist effect *in vivo*, in a rat model of rotational locomotion, by administering directly into the caudate nucleus of the brain a peptide comprising a fragment of one of the transmembrane amino acid sequences of the D2 dopamine receptor. Most importantly, the inventors have demonstrated specificity, with no disruption of other closely related receptors by the peptide designed for the D2 dopamine receptor.

In a further animal model, the inventors have shown that a peptide comprising a portion of a transmembrane domain of the β1-adrenergic receptor inhibited the function of that receptor, and a peptide comprising a portion of a transmembrane domain of the α1A-adrenergic receptor inhibited the function of that receptor, as evidenced by the effect of these peptides on cardiac function and blood pressure.

The models described herein are not, however, limited to GPCRS. The inventors have shown, for example,

that specific antagonists can be prepared, in accordance with the invention, for tyrosine kinase receptors and immune receptors.

Antagonist peptides in accordance with the invention have also been demonstrated to interfere with the function of mammalian receptors employed by viruses to attack mammalian cells, as exemplified by the inhibition of HIV infectivity by peptides derived from the TM domains of the CCR5 and CD4 receptors.

In accordance with a further embodiment, the inventors have shown the applicability of the invention to prokaryotic systems, with the demonstration of the anti-bacterial effect of antagonist peptides derived from a bacterial energy-dependent transporter.

Antagonist peptides in accordance with the invention may be prepared by any suitable peptide synthetic method.

Chemical synthesis may be employed, for example standard solid phase peptide synthetic techniques may be used. In standard solid phase peptide synthesis, peptides of varying length can be prepared using commercially available equipment. This equipment can be obtained from Applied Biosystems (Foster City, CA.). The reaction conditions in peptide synthesis are optimized to prevent isomerization of stereochemical centres, to prevent side reactions and to obtain high yields. The peptides are synthesized using standard automated protocols, using t-butoxycarbonyl-alpha-amino acids, and following the manufacturer's instructions for blocking interfering groups, protecting the amino acid to be reacted, coupling, deprotecting and capping of unreacted residues. The solid support is generally based on a polystyrene resin, the resin acting both as a support for the growing peptide chain, and as a protective group for the carboxy terminus. Cleavage from the resin yields the

free carboxylic acid. Peptides are purified by HPLC techniques, for example on a preparative C18 reverse phase column, using acetonitrile gradients in 0.1% trifluoroacetic acid, followed by vacuum drying.

Antagonist peptides may also be produced by recombinant synthesis. A DNA sequence encoding the desired peptide is prepared, for example by cloning the required fragment from the DNA sequence encoding the complete receptor, obtainable from genomic DNA or from commercially available genomic or cDNA libraries, and subcloning into an expression plasmid DNA. Suitable mammalian expression plasmids include pRC/CMV from Invitrogen Inc. The gene construct is expressed in a suitable cell line, such as a Cos or CHO cell line and the expressed peptide is extracted and purified by conventional methods. Suitable methods for recombinant synthesis of peptides are described in "Molecular Cloning" (Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory Press, 1989).

Analogues of a transmembrane amino acid sequence of an integral membrane protein may be prepared by similar synthetic methods. The term "analogue" extends to any functional and/or chemical equivalent of a transmembrane amino acid sequence and includes peptides having one or more conservative amino acid substitutions, peptides incorporating unnatural amino acids and peptides having modified side chains.

Examples of side chain modifications contemplated by the present invention include modification of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidation with methylacetimidate; acetylation with acetic anhydride; carbamylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6,

trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2, 3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via -acylisourea formation followed by subsequent derivatisation, for example, to a corresponding amide.

Sulfhydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; carbamylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides. Tyrosine residues may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodacetic acid derivatives of N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not

limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid-, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers or amino acids.

Examples of conservative amino acid substitutions are substitutions within the following five groups of amino acids (amino acids are identified by the conventional single letter code): Group 1: F Y W; Group 2: V L I; Group 3: H K R; Group 4: M S T P A G; Group 5: D E.

Fragments or analogues of the antagonist peptides of the invention may be conveniently screened for their effectiveness as receptor antagonists, for example by examining their ability to inhibit ligand-binding by the relevant receptor which has been pre-incubated with the peptide. Ligand-binding inhibition can be determined, for example, by a soluble receptor radioligand binding assay, as described herein.

The antagonist peptides may also be screened for their effectiveness as receptor antagonists by examining their ability to impair receptor coupling to second messenger systems or their ability to impair some functional activity. For example, for a GPCR such as the D2 dopamine receptor, the ability of the antagonist to block D2 receptor mediated attenuation of adenylyl cyclase activity provides a convenient index of efficacy as described herein.

For a tyrosine kinase receptor such as the EGF receptor, the ability of antagonists to inhibit EGF receptor tyrosine phosphorylation, as described herein, provides an index of efficacy.

The peptide antagonists of the invention may be administered therapeutically by injection or by oral,

nasal, buccal, rectal, vaginal, transdermal or ocular routes in a variety of formulations, as is known to those in the art.

For oral administration, various techniques can be used to improve stability, based for example on chemical modification, formulation and use of protease inhibitors. Stability can be improved if synthetic amino acids are used, such as peptoids or betidamino acids, or if metabolically stable analogues are prepared.

Formulation may be, for example, in water/oil emulsion or in liposomes for improved stability. Oral administration of peptides may be accompanied by protease inhibitors such as aprotinin, soybean trypsin inhibitor or FK-448, to provide protection for the peptide. Suitable methods for preparation of oral formulations of peptide drugs have been described, for example, by Saffran et al., 1979) (use of trasylol protease inhibitor); Lundin et al. (1986) and Vilhardt et al., (1986).

Due to the high surface area and extensive vascular network, the nasal cavity provides a good site for absorption of both lipophilic and hydrophilic drugs, especially when coadministered with absorption enhancers. The nasal absorption of peptide-based drugs can be improved by using aminoboronic acid derivatives, amastatin, and other enzyme inhibitors as absorption enhancers and by using surfactants such as sodium glycolate, as described in Amidon et al., (1994).

The transdermal route provides good control of delivery and maintenance of the therapeutic level of drug over a prolonged period of time. A means of increasing skin permeability is desirable, to provide for systemic access of peptides. For example, iontophoresis can be used as an active driving force for charged peptides or

chemical enhancers such as the nonionic surfactant n-decylmethyl sulfoxide (NDMS) can be used.

Transdermal delivery of peptides is described in Amidon et al. (1994) and Choi et al. (1990).

Peptides may also be conjugated with water soluble polymers such as polyethylene glycol, dextran or albumin or incorporated into drug delivery systems such as polymeric matrices to increase plasma half-life.

More generally, formulations suitable for particular modes of administration of peptides are described, for example, in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Company (Easton, PA.)

The peptide antagonists of the invention also provide a tool for the elucidation of the function of many important orphan receptors whose structures and locations are known but for which the endogenous ligand is unknown. Disruption of the function of an orphan receptor by a transmembrane peptide antagonist and observation of the resulting loss or disruption of function will assist in elucidating the role of the orphan receptor.

The present invention also provides new methods of tissue imaging. An antagonist peptide derived from the transmembrane amino acid sequence of a membrane-spanning protein may be labelled with a suitable signalling moiety, such as an imaging radionuclide, and administered *in vivo*. The labelled peptide binds stably to the receptor permitting visualisation and quantification of the receptor. Suitable radionuclides include technetium^{99}, thallium, ^{11}C or ^{18}F.

Peptides may be labelled by conventional methods known to those skilled in the art.

The specificity of the antagonist peptides of the invention for the receptor will provide improved accuracy

and precision in the determination of receptor localisation and receptor density on cells and in tissues. Detection of the signalling moiety and therefore of the bound receptor can be carried out by conventional methods suitable for each particular labelling moiety.

In a further embodiment, the invention provides new methods for gene therapy utilising a genetically engineered, recombinant nucleotide sequence encoding a peptide antagonist, incorporated in a suitable transfection vector for introduction of the coding sequence into a selected cell or tissue, either *ex vivo* or *in vivo*, in order to provide for *in vivo* production of a selected integral membrane protein antagonist. For example, adenovirus and vaccinia virus are employed as vectors for gene therapy. Gene therapy techniques are reviewed, for example, in (Hanania, E.G. (1995), *Am. J. of Med.*, v. 99, pp. 537-552). A recombinant nucleotide sequence encoding an antagonist peptide of the invention may be incorporated into a transfection vector under the control of a tissue-specific promoter which ensures expression of the nucleotide sequence only in the selected target tissue. For example, a viral vector may be employed incorporating a promoter which directs expression only in brain cells which have dopamine D2 receptors and a nucleotide sequence encoding an antagonist peptide specific for the dopamine D2 receptor. For treatment of a disorder associated with over-activity of dopamine receptors, the viral preparation can be introduced directly into the brain, for example by intra-cerebroventricular injection or infusion, where the virus is taken up by brain cells, but the peptide is produced only where required. Non-viral gene therapy methods are described, for example, in EP 289034.

In a further embodiment, the invention provides transgenic animal models expressing transmembrane peptide antagonists which modulate endogenous integral membrane protein function. These animal models will provide a tool for testing the design, efficacy and toxicology of integral membrane protein antagonist peptides and will also provide models that mimic clinical diseases.

Transgenic animal models in accordance with the invention can be created by introducing a DNA sequence encoding a selected peptide antagonist either into embryonic stem (ES) cells of a suitable animal, for example a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte microinjection.

The ES cells are inserted into a young embryo and this embryo or an injected oocyte are implanted into a pseudo-pregnant foster mother to grow to term.

The techniques for generating transgenic animals are now widely known and are described in detail, for example, in Hogan et al., (1986), and M. Capecchi (1989).

1. PEPTIDE ANTAGONISTS FOR G-PROTEIN COUPLED RECEPTORS

The G-protein coupled receptors have a common pattern of seven hydrophobic membrane-spanning domains. These receptors are involved in a wide variety of pathways. Table 1 lists the various receptors which belong to this superfamily.

(a) Dopamine Receptor Antagonists

In accordance with one embodiment of the present invention, dopamine receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected dopamine receptor are provided.

Five distinct dopamine receptors have been identified and are designated D1 to D5 (Seeman, P.

(1995). All belong to the family of G protein-coupled receptors (GPCRs) that have seven highly conserved membrane spanning regions which are linked by intracellular and extracellular loops (O'Dowd, 1993). Comparison of the primary structure of GPCRs shows that the greatest similarity exists in the transmembrane domains, whereas greatest differences are found within the N- and C-terminal regions and the cytoplasmic third loop connecting transmembrane domains V and VI. For instance, D1 and D5 receptors have a shorter third intracellular loop and a longer carboxyl tail compared to D2, D3, and D4 receptors.

One of ordinary skill in the art is enabled by this invention to identify specific antagonist compounds which regulate or inhibit each of the dopamine receptors.

(i) Dopamine D2 receptor antagonists

The following description of the dopamine D2 receptor and of peptide antagonists of that receptor provides a general illustration of the selection of a specific antagonist peptide to an integral membrane protein, in accordance with the invention. The same method may be applied by one of ordinary skill in the art to select an antagonist to any selected integral membrane protein.

The dopamine D2 receptor is activated by the neurotransmitter, dopamine, leading to the inhibition of intracellular adenylate cyclase.

The D2 receptor gene encodes a long and a short form of the receptor, differing by a 29 amino acid segment in the third intracellular loop. The long and short forms have identical transmembrane domains.

Figure 1 shows a two-dimensional representation of the D2 dopamine receptor spanning the cell membrane. The seven transmembrane domains and two cytoplasmic domains

are identified, the transmembrane amino acid sequences being boxed.

Table 2 shows the information available in the SwissProt database for the dopamine D2 receptor, Accession No. P14416 (Sequence ID NO:8).

The transmembrane (TM) domains are identified by amino acid number, as follows:

TM 1	amino acids 38 to 60
TM 2	amino acids 72 to 97
TM 3	amino acids 109 to 130
TM 4	amino acids 152 to 174
TM 5	amino acids 187 to 210
TM 6	amino acids 374 to 397
TM 7	amino acids 406 to 429

The amino acid sequences of these transmembrane domains can be determined from the complete amino acid sequence provided. Any one of these transmembrane amino acid sequences may be selected for use as a specific antagonist of the D2 dopamine receptor. A D2 dopamine receptor antagonist peptide may, therefore, be selected from the following transmembrane amino acid sequences:

TM I ATLLTLLIAVIVFGNVLVCMAVS (Sequence ID NO:1)
TM II LIVSLAVADLLVATLVMPWVVYLEVV (Sequence ID NO:2)
TM III IVFTLDVMMCTASILNLCAISI (Sequence ID NO:3)
TM IV VTVMISIVWVLSFTISCPLLFGL (Sequence ID NO:4)
TM V PAFVVYSSIVSFYVPFIVTLLVYI (Sequence ID NO:5)
TM VI MLAIVLGVFIICWLPFFITHILN (Sequence ID NO:6)
TM VII VLYSAFTWLGYVNSAVNPIIYTTF (Sequence ID NO:7)

or may be an effective fragment or analogue of any of these sequences.

D2 receptors are found in brain, where the highest densities have been found in the striatum (Caudate-putamen, nucleus accumbens), olfactory tubercle, and substantia nigra and pituitary, whereas lower densities

are present in the cortex, hippocampus and limbic brain regions (Bouthenet et al., 1987, 1991, Mansour et al., 1990). At the cellular level, D2-like receptors have been identified on synaptic nerve terminals, and there is evidence that D2 receptors are colocalized with D1 receptors in certain neuronal populations (Surmeier et al., 1992). These mapping studies also indicate a pre-synaptic localization of D2-like receptors where they may function as autoreceptors regulating the synthesis and/or release of dopamine (Starke et al., 1989, Sokoloff et al., 1990).

In accordance with the invention, the following peptides based on the D2 dopamine receptor TM domains have been synthesised: D2-TM I: YATLLTLLIAVIVFGNVLVC (Sequence ID NO:61);

D2-TM II: VSLAVADLLVATLVMPWVVY (Sequence ID NO:60);
D2-TMIII:TLDVMMCTASILNLCAISID (Sequence ID NO:59);
D2-TM IV: RVTVMISIVWVLSFTISCPL (Sequence ID NO:58);
D2-TM V: PAFVVYSSIVSFYVPFIVTL (Sequence ID NO:57);
D2-TM VI: LAIVLGVFIICWLPFFITHI (Sequence ID NO:56);
D2-TM VII: LYSAFTWLGYVNSAVNPIIY (Sequence ID NO:55);
D2-TM VII: TWLGYVNSA (Sequence ID NO:64).

As described in Examples 1 to 3, the inventors have shown that peptides of amino acid sequence corresponding to all or a portion of a D2 receptor TM domain are highly specific antagonists of D2 receptor binding and function *in vitro* and *in vivo*.

(ii) Dopamine D1 and D3 to D5 receptor antagonists

The dopamine D1 and D3 to D5 receptors are also receptors for the neurotransmitter dopamine and are found in brain as well as in other tissues.

The amino acid sequences of these receptors, and identification of their transmembrane domains, can be obtained, for example, from SwissProt Database under the

Accession Numbers listed in Table 1.

Using this information, one skilled in the art can readily ascertain the transmembrane amino acid sequences for each of these dopamine receptors and can thereby synthesise antagonist peptides suitable and specific for each receptor. For example, for the D1 dopamine receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

TM 1: ILTACFLSLLILSTLLGNTLVCAAV (Sequence ID NO:9);

TM 2: FFVISLAVSDLLVAVLVMPWKAVAEIA (Sequence ID NO:10);

TM 3: NIWVAFDIMCSTASILNLCVISVD (Sequence ID NO:11);

TM 4: AAFILISVAWTLSVLISFIPVQLSW (Sequence ID NO:12);

TM 5: TYAISSSVISFYIPVAIMIVTYTRI (Sequence ID NO:13);

TM 6: TLSVIMGVFVCCWLPFFILNCILPFC (Sequence ID NO:14);

TM 7: FDVFVWFGWANSSLNPIIYAFNAD (Sequence ID NO:15).

The D1 dopamine receptor has been associated with drug abuse and the D3 and D4 receptors have been associated with schizophrenia. Antagonists of these receptors in accordance with the invention provide specific therapeutic agents for use in these conditions.

(b) Adrenergic Receptor Antagonists

In accordance with a further embodiment of the invention, adrenergic receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected adrenergic receptor are provided.

The adrenergic receptors (AR) mediate the effects of the catecholamines epinephrine and norepinephrine on a wide variety of physiologic processes, such as regulation of blood pressure and heart rate.

Molecular cloning studies have now shown that the physiological actions of epinephrine and norepinephrine are mediated by the products of at least nine types of AR. These receptors can be subdivided into three groups,

each capable of coupling to different G proteins. β_1-, β_2-, and β_3AR are involved in the activation of adenylyl cyclase. Conversely, activation of the platelet and kidney α_2AR (α_2AR-C10 and α_2AR-C4, respectively) inhibits adenylyl cyclase activity via the intermediacy of the G protein G_i. The α_1 AR receptors (α_{1A} and α_{1B}) have the ability to stimulate phospholipase C. Stimulation of this effector enzyme leads to membrane phospholipid hydrolysis and the subsequent mobilization of calcium from intracellular stores.

The various AR, and the respective G proteins to which they couple, provide the means by which the two adrenergic agonists epinephrine and norepinephrine can elicit many different intracellular responses.

For example, for the β1-adrenergic receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

TM I : GMGLLMALIVLLIVAGNVLVIVAI (Sequence ID NO:16);
TM II: IMSLASADLVMGLLVVPFGATIVV (Sequence ID NO:17);
TM III:ELWTSVDVLCVTASIETLCVIALD (Sequence ID NO:18);
TM IV: RGLVCTVWAISALVSFLPILMHWW (Sequence ID NO:19);
TM V : RAYAIASSVVSFYVPLCIMAFVYL (Sequence ID NO:20);
TM VI: LGIIMGVFTLCWLPFFLANVVKAF (Sequence ID NO:21);
TM VII:RLFVFFNWLGYANSAFNPIIYCRS (Sequence ID NO:22).

For example, for the β2-adrenergic receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

TM I: TAGMGLLMALIVLLIVAGNVLVIVAI(Sequence ID NO:65);
TM II: LFIMSLASADLVMGLLVVPFGATIVV(Sequence ID NO:66);
TM III: WTSVDVLCVTASIETLCVIALD(Sequence ID NO:67);

TM IV: ARGLVCTVWAISALVSFLPILMHW (Sequence ID NO:68);
TM V: RAYAIASSVVSFYVPLCIMAFVYL (Sequence ID NO:69);
TM VI: TLGIIMGVFTLCWLPFFLANVVKA (Sequence ID NO:70);
TM VII: DRLFVFFNWLGYANSAFNPIIYC (Sequence ID NO:71).

For example, for the α1A-adrenergic receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

TM I: GVGVGFLAAFILMAVAGNLLVILSV (Sequence ID NO:23);
TM II: FIVNLAVADLLLSATVLPFSATMEVL (Sequence ID NO:24);
TM III: DVWAAVDVLCCTASILSLCTISV (Sequence ID NO:25);
TM IV: AAILALLWVVALVVSVGPLLGWKEP (Sequence ID NO:26);
TM V: AGYAVFSSVCSFYLPMAVIVVMYC (Sequence ID NO:27);
TM VI: LAIVVGVFVLCWFPFFFVLPLGSL (Sequence ID NO:28);
TM VII: EGVFKVIFWLGYFNSCVNPLIYPCS (Sequence ID NO:29).

The inventors have shown that the peptides FFNWLGYANSAFNP (Sequence ID NO:30) and GYANSAFNP (Sequence ID NO:72), both fragments of the TM VII domain of the human β1-adrenergic receptor, inhibited the function of that receptor *in vitro* and *in vivo*, as described in Examples 4 and 5.

The inventors have also shown that the peptides VFKVIFWLGYFNSCVN (Sequence ID NO:31) and VFKVIFWLGYFNS (Sequence ID NO:73), both fragments of the TM VII domain of the human α1A-adrenergic receptor, inhibited the function of that receptor *in vivo*, as shown in Example 5.

Adrenergic receptor antagonists are accepted therapeutic agents for treatment of hypertension. The adrenergic receptor antagonist peptides of the present invention provide new agents with previously unavailable specificity for use in treatment of hypertension.

(c) Adenosine Receptor Antagonists

In accordance with a further embodiment of the

invention, adenosine receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected adenosine receptor are provided.

Adenosine is a neuromodulator which is released in response to increased activity or stress.

Adenosine receptors are found in both central and peripheral neural locations. Four subtypes of adenosine receptors, designated A1, A2a, A2b and A3, have been identified.

In general, adenosine exerts a depressant action in the brain, heart and kidneys by activating adenosine receptors. The depressant action in the brain is believed to confer neuroprotection. Moreover, centrally acting adenosine has been shown to be involved in pain, cognition, movement and sleep. Peripherally, adenosine is believed to have arrhythmic, hypotensive and antilipolytic properties.

The antiasthmatic effects of theophylline and the antidepressant and cognition-enhancing effects of caffeine are attributed to their action as adenosine receptor antagonists.

Adenosine receptor antagonists have a role as therapeutics in the treatment of cardiovascular, renal and central nervous system disorders and are likely to be useful as anti-asthmatics, anti-depressants, anti-arrhythmics, anti-Parkinsonian therapeutics, cognitive enhancers and as renal protective agents.

(d) Vasopressin type 2 receptor antagonists

In accordance with a further embodiment of the invention, vasopressin type 2 receptor antagonist peptides and a method for regulating or inhibiting the activity of the receptor are provided.

The vasopressin type 2 (V2) receptor of the kidney collecting tubules binds arginine vasopressin, leading to

G protein-mediated activation of adenylate cyclase and decreased water permeability of the tubule cells, resulting in fluid retention by the kidney. Defects in the V2 receptor are a cause of congenital nephrogenic *diabetes insipidus*, characterized by excessive urine excretion (polyuria) and failure to concentrate urine in response to vasopressin.

Currently there are few selective pharmaceutical agents that target this important receptor protein.

For the V2 receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

TMI: AELALLSIVFVAVALSNGLVLAALA (Sequence ID NO:74);
TMII: IGHLCLADLAVALFQVLPQLAW (Sequence ID NO:75);
TMIII: AVKYLQMVGMYASSYMILAMTL (Sequence ID NO:76);
TMIV: VLVAWAFSLLLSLPQLFIFAQ (Sequence ID NO:77);
TMV: TYVTWIALMVFVAPTLGIA (Sequence ID NO:78);
TMVI: MTLVIVVVYVLCWAPFFLVQLW (Sequence ID NO:79);
TMVII: LLMLLASLNSCTNPWIYASF (Sequence ID NO:80).

Antagonist peptides based on the amino acid sequence of the TM domains of the V2 Receptor, in accordance with the invention, provide therapeutic agents which can reduce or prevent the function of that receptor, providing a potent diuretic for the management of hypertension, congestive heart failure and other fluid retentive disorders.

The inventors have shown that the peptide LMLLASLNSCTNPWIY (Sequence ID NO:53), a fragment of the TM VII domain of the V2 receptor, by inhibition of the V2 receptor, acted as a diuretic in the intact rat, as described in Example 6.

(e) Chemokine receptor antagonists

In accordance with a further embodiment of the

invention, chemokine receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected chemokine receptor are provided.

The cell surface receptors for chemokines belong to the family of G protein coupled receptors, and have been implicated in a number of physiological functions.

One of the major steps leading to HIV infection of mammalian cells involves virus interaction with two receptors belonging to the family of G protein coupled receptors, namely (i) the cell surface single TM-spanning CD4 receptor and (ii) the seven-TM chemokine receptors (such as CCR5 and CXCR4, also called fusin), in order for the virus to gain access to the cell. The chemokine receptors, CCR5 and CXCR4, have been identified as the main cofactors necessary for HIV entry into CD4-positive cells(Dragic et al., (1996); Weiss et al., (1996)). The phenotype of the HIV virus determines whether it preferentially uses CXCR4 or CCR5, or sometimes both receptors, for entry into CD4-positive cells. CCR2B and CCR3 are minor co-factors for HIV entry.

(i) Antagonists for the CCR5, CXCR4, CCR2B and CCR3 receptors

For the CCR5 receptor or the CXCR4 receptor, an antagonist peptide may be selected from the transmembrane amino acid sequences of these receptors shown in Table 4 or may be an effective fragment or analogue of any of these sequences. Similarly, an antagonist peptide based on the transmembrane domain of the CCR2B or CCR3 receptor can be used to block HIV entry, as these receptors may also act as coreceptors with the CD4 receptors for HIV virus entry.

The inventors have shown that the peptide LYSLVFIFGFVGN (Sequence ID NO:82), a fragment of the TM I domain of the CCR5 receptor, inhibited HIV infection of

human PBMC cells, as described in Example 7.

An antagonist peptide based on the amino acid sequence of any one of the TM domains of the CCR5 receptor, the CXCR4 receptor or the CCR2B or CCR3 receptors, in accordance with the invention, provides a therapeutic agent which can disrupt the function of the respective receptor, thereby preventing entry of the HIV virus into CD4-positive cells containing that receptor. Such therapeutic agents may be used prophylactically or after exposure to the HIV virus.

(f) Serotonin Receptor Antagonists

In accordance with a further embodiment of the invention, serotonin receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected serotonin receptor are provided.

The diverse biological activities of the neurotransmitter, serotonin, are mediated through a variety of serotonin receptors now numbering 15. All except the 5HT3 receptor belong to the superfamily of GPCRs.

Serotonin has been associated with a number of neuropsychiatric disorders such as depression, consummatory disorders and drug addiction, migraine and other vascular disorders.

For example, for the 5-HT1A serotonin receptor, an antagonist peptide may be selected from the transmembrane amino acid sequences of the receptor shown in Table 4 or may be an effective fragment or analogue of any of these sequences.

An antagonist peptide based on the amino acid sequence of a transmembrane domain of any serotonin receptor, in accordance with the invention, provides a therapeutic agent which can disrupt the function of that receptor and hence can be used for specific directed therapy in

neuropsychiatric disorders such as depression, consummatory disorders and drug addiction, migraine and other vascular disorders.

(g) Mu-opioid Receptor Antagonists

In accordance with a further embodiment of the invention, opioid receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected opioid receptor are provided.

The diverse biological activities of the endogenous opioid peptides are mediated through a variety of opioid receptors including Mu, Delta, and Kappa.

Opioid neuronal systems play important roles in a wide variety of physiological processes including pain, mood, learning, thermoregulation, ingestive behaviour, motor activity and the perception of reward, with critical effects in modulating endocrine, cardiovascular, respiratory, gastrointestinal, autonomic and immune functions.

Currently there are few selective pharmaceutical agents that target these important receptor proteins.

For example, for the Mu opioid receptor (MOR), an antagonist peptide may be selected from the transmembrane amino acid sequences of the receptor shown in Table 4 or may be an effective fragment or analogue of any of these sequences.

An antagonist peptide based on the amino acid sequence of the transmembrane domains of the Mu-opioid receptor, in accordance with the invention, provides a therapeutic agent which can disrupt the function of that receptor and can be used in the treatment of disorders including substance abuse, obesity, eating disorders and bowel motility.

(h) Angiotensin Type 1 Receptor Antagonists

In accordance with a further embodiment of the

invention, angiotensin receptor antagonist peptides and a method for regulating or inhibiting the activity of a selected angiotensin receptor are provided.

Angiotensin II (ANG II), a component in the renin-angiotensin system (RAS), is an important factor in the pathogenesis of cardiovascular diseases including hypertension, cardiac left ventricular hypertrophy (LVH) and congestive heart failure. Angiotensin II also contributes to structural alterations of the vasculature such as medial hypertrophy, neointima formation and post-infarct remodeling of the heart. The biological activities of angiotensin II are mediated by the ANG II AT1 and AT2 receptor subtypes which display a heterogeneous distribution. Virtually all known physiological and cardiovascular actions of ANG II have been attributed to the AT1 receptor which is coupled to a G-protein, while stimulation of AT2 receptors, which are not G-protein-coupled, leads to an inhibition of cell proliferation and possibly induces cell differentiation. It is conceivable that under physiological conditions AT1 receptors facilitate, whereas AT2 receptors inhibit, angiogenesis. Under pathophysiological conditions, such as postmyocardial infarction or LVH, the AT2 receptor could be upregulated to control excessive growth mediated in part by the AT1 receptor.

For example, for the ANG II AT1 receptor, an antagonist peptide may be selected from the transmembrane amino acid sequences of the receptor shown in Table 4 or may be an effective fragment or analogue of any of these sequences.

Blockers of the angiotensin system are currently used in hypertension, congestive heart failure and diabetic neuropathy. Antagonist peptides based on the amino acid sequence of a TM domain of the AT1 receptor

provide therapeutic agents which can be used in these same diseases.

(i) Neuropeptide Y 5 Receptor Antagonists

In accordance with a further embodiment of the invention, neuropeptide Y5 (NPY5) receptor antagonist peptides and a method for regulating or inhibiting the activity of that receptor are provided.

Neuropeptide Y (NPY) plays important roles in the central control of appetite and energy balance. These specific activities are mediated by the NPY5 receptor. The amino acid sequence deduced from rat Y5 cDNA shows only 30-33% identity to other NPY receptors, including Y1, Y2, and Y4/PP1. Pharmacological analysis shows that the Y5 receptors have high affinity for the peptides that elicit feeding (e.g. NPY, PYY, (2-36)NPY, and (LP)NPY) and low affinity for nonstimulating peptides (e.g. (13-36)NPY and rat PP)(Gerald et al.(1996), Nature, **382**,168).

Antagonist peptides based on the amino acid sequence of the TM domains of the NPY5 receptor, provide therapeutic agents which can be used for the management of appetite regulation in obesity, and type 2 diabetes mellitus and related conditions.

(j) Melanocyte Stimulating Hormone Receptor

In accordance with a further embodiment of the invention, melanocyte stimulating hormone (MSH) antagonist peptides and a method for regulating or inhibiting the activity of a selected MSH are provided.

MSH is a strong stimulator of pigment cells, modulating skin colour change in some animals. MSH has also been shown to act as a neurotransmitter in the central nervous system, as an endocrine stimulant and as a modulator of immune inflammatory responses. The hormone is considered a potential tool in the diagnosis

and therapy of melanoma, as it has been used in conjugation with cytotoxin and cytotoxic T-cells for killing melanoma cells by recognizing their MSH receptors.

Antagonist peptides based on an amino acid sequence of a TM domain of the MSH receptor, as shown in Table 4, provide therapeutic agents which can be used for the management of hyperpigmentation, melanoma and inflammatory disorders.

2. PEPTIDE ANTAGONISTS OF TYROSINE KINASE RECEPTORS

In accordance with a further embodiment of the invention, tyrosine kinase antagonist peptides and a method for regulating or inhibiting the activity of a selected tyrosine kinase receptor are provided.

The tyrosine kinase receptors have an amino terminus involved in ligand binding, a single membrane-spanning domain and a homologous carboxyl tail catalytic domain with intrinsic tyrosine kinase activity (Kraus et al., 1989).

Examples of such tyrosine kinase receptors include receptor families for a number of growth factors, including epidermal growth factor (EGF), colony-stimulating factor 1/platelet derived growth factors and insulin/insulin-like growth factor , *fibroblast growth factor, tumor necrosis factor, vascular endothelial growth factor.* Tyrosine kinase receptors are localized in a wide range of epithelial and fibroblastic cells. Tyrosine kinase receptors mediate a plethora of biological activities, including regulation of cell proliferation, angiogenesis and apoptosis among others.

Binding of ligand to the extracellular portion of a tyrosine kinase receptor results in an association of two receptor molecules (dimerization) that leads to

conformational changes resulting in the phosphorylation of the cytoplasmic domain of the receptor (Boni-Schnetzler et al., 1987).

(a) Epidermal growth factor receptor antagonists

The epidermal growth factor (EGF) receptor has four subtypes, identified as erbl to erb4. A schematic representation of an EGF receptor, with a single membrane-spanning domain, is shown in Figure 2. The family of EGF receptors is prototypic for the other tyrosine kinase receptors.

EGF receptors have been shown to act as oncogenes by mechanisms of overexpression, or mutations that constitutively activate the intrinsic tyrosine kinase activity of these proteins (Schlessinger, J., 1986; Yarden et al., 1987). In particular, the EGF-erb3 receptor is overexpressed in a subset of human mammary tumors.

The ability to inhibit or regulate activity of the EGF family of receptors by the antagonist peptides of the invention provides a new, specific tool to prevent the development of, or control, of neoplastic growth in psoriasis and cancer.

For example, for the EGF-erbl receptor an antagonist peptide may have the transmembrane domain amino acid sequence IATGMVGALLLLLVVALGIGLFM (Sequence ID NO:32) or may be an effective fragment or analogue of that sequence, and for EGF-erb3, the antagonist may be MALTVIAGLVVIFMMLGGTFL (Sequence ID NO:83) or an effective analogue or fragment thereof.

The inventors have shown that the peptide LTVIAGLVVIF (Sequence ID NO:84), a fragment of the TM domain of the EGF-erb3 receptor, inhibited the tyrosine kinase function of that receptor in cultured A431 cells, as described in Example 8. Such an antagonist peptide

provides a therapeutic agent for inhibition of cell proliferation, for use , for example, in cancer, psoriasis or hyper-keratotic disorders.

(b) Fibroblast Growth Factor receptor antagonists

Tyrosine kinase receptors such as Fibroblast Growth Factor receptor, FGFr (also belonging to the immunoglobulin superfamily), Vascular Endothelial Growth Factor receptor, VEGFr (also belonging to the immunoglobulin superfamily), and Platelet Derived Growth Factor receptor, PDGFr, play an important role in angiogenesis. Angiogenesis comprises the processes leading to the generation of new blood vessels through sprouting from already-existing blood vessels. Blood vessel growth is associated with wound healing, tissue growth and repair; abnormal angiogenesis occurs in pathologies such as cancer and diabetic retinopathy.

Angiogenic inhibitors are of clinical significance because they can be used to influence directly the angiogenic processes involved, for example, in wound healing. Angiogenesis inhibitors will also be of value in treatment of diseases including pathogenic neovascularization such as Kaposi's sarcoma, diabetic retinopathy, and malignant tumor growth.

The Fibroblast Growth Factor receptor (FGFr) has two forms, identified as FGFr1 and FGFr2.

Antagonist peptides based on the amino acid sequence of the TM domain of the FGFr1 (IIIYCTGAFLISCMVGSVIVY: Sequence ID NO:85) or FGFr2 (AIYCIGVFLIACMVVTVILC: Sequence ID NO:86) receptors, provide therapeutic agents which can be used to regulate angiogenesis.

(c)Vascular Endothelial Growth Factor receptor antagonists

In accordance with a further embodiment of the invention, vascular endothelial growth factor receptor 1

and 2 antagonist peptides and a method for regulating or inhibiting the activity of the selected receptor are provided.

The Vascular Endothelial Growth Factor receptor (VEGFr) has two forms, identified as VEGFr1 and VEGFr2, with TM domains ISYSFQVARGMEFLSSRKCIH (Sequence ID NO:87) and IIILVGTTVIAMFFWLLLVIILGTV (Sequence ID NO:88), respectively.

(d) Trk A receptor antagonists

Neurotrophins act by binding and activating membrane receptors which belong to the trk family of protein tyrosine kinases. Trk A is the receptor for nerve growth factor, Trk B for brain derived neurotrpoc factor(BDNF) and neurotrophin-4, and trk C for the neurotrophin-3 receptor. Trk B and trk C are abundantly expressed in different parts of developing and adult brain. Like other members of receptor tyrosine kinase family, trk A receptors are activated by ligand- induced dimerization and autophosphorylation, which triggers the cellular responses through the activation of the ras -MAP-kinase pathway. Trk B and C, (but not trk A) are alternatively spliced into two different types of receptor isoforms: the full-length, tyrosine kinase (TK) domain-containing form TK(+) and the truncated form TK(-). These isoforms are identical in their extracellular and transmembrane domains, but in place of the intracellular TK domain, TK(-) forms only contain short unique intracellular tail regions. Both receptor variants bind neurotrophins, but only TK(+) can activate intracellular signal transduction.

For example, for the Trk A receptor, an antagonist peptide may have the transmembrane domain amino acid sequence AVFACLFLSTLLLVI (Sequence ID NO:89) or may be an effective fragment or analogue thereof.

Antagonist peptides based on the amino acid sequence of the transmembrane domain of the trkA receptor provide therapeutic agents which can be used to reduce or inhibit nerve growth factor activity.

3. **Ion Channel and Channel Protein Antagonists**

A number of integral membrane proteins, including the energy-dependent transporter pumps, form ion channels or ion channel receptors or are channel proteins.

(a) Bacterial Energy-dependent Transporter Antagonists

In accordance with a further embodiment of the invention, bacterial energy-dependent transporter antagonist peptides and a method for regulating or inhibiting the activity of the selected receptor are provided.

The integrity of bacterial membranes is maintained by a variety of membrane proteins, including bacterial ATPase transporter. Disrupting the function of the critical membrane protein may lead to loss of bacterial cell viability. The energy derived from metabolic processes in bacteria is used to generate ionic gradients across the cytoplasmic membrane. The ion-translocating enzyme, F_1F_0 ATPase, synthesizes ATP using a proton gradient and is the enzyme responsible for oxidative phosphorylation. The energy of the proton gradient drives ATP synthesis, catalyzed by the F_1F_0 ATPase. The *E. coli unc* operon, which codes for the ATPase, contains nine genes coding for the F_o and F_1 domains of the enzyme. The F_o portion is membrane-intrinsic and has three sub-units, a, b and c.

The amino acid sequence of the Gram negative Fo c subunit of the *E. coli* ATPase F_1F_0 is shown in Kanazawa et al. (1981) Biochemical and Biophysical Research Communications v. 103, pp. 613-620.

As an example of antagonist peptides for a bacterial energy-dependent transporter, an antagonist peptide for the E. coli F_1F_o ATPase may have the Fo b sub-unit transmembrane sequence MAAAVMMGLAAIGAAIGIGILGG (Sequence ID NO:90) or the Fo c sub-unit TM sequence NATILGQAIAFVLFVLFCM (Sequence ID NO:91) or may be an effective fragment or analogue of one of these sequences.

The inventors have shown that peptide GQAIAFVLFVL (Sequence ID NO:92), based on the amino acid sequence of the TM domain of the Gram negative ATPase Fo b subunit, and peptide LAAIGAAIGIGIL (Sequence ID NO:93), based on the Fo c subunit, used alone or in combination, could prevent the growth of *E. coli*, as described in Example 9.

Antagonist peptides based on the amino acid sequence of a TM domain of the Gram negative ATPase Fo b subunit or Fo c subunit, provide therapeutic agents which can be used as anti-bacterials.

(b) Mammalian Energy-dependent Transporters

In accordance with a further embodiment of the invention, mammalian energy-dependent transporter peptides and a method for regulating or inhibiting the activity of the selected transporters are provided.

P-glycoprotein or MDR1 protein, is an example of a mammalian energy-dependent transporter. It is an energy--dependent efflux pump responsible for drug efflux and decreased drug accumulation in multi-drug resistant (MDR) cells.

The activation of the mdr1 gene which encodes the protein can occur under various types of stimulation, including under the effect of anti-cancer drugs. P-glycoprotein is an ATPase transporter which is believed to extrude xenobiotics from the plasma membrane rather than from the cytoplasm. Although potential sites

of interaction of P-glycoprotein with its various ligands have been identified, especially at the level of putative transmembrane domains, the exact mechanism for drug pumping has never been elucidated. It is desirable to have therapeutic agents which are able to reverse the multi-drug resistant state but are devoid of any pharmacological properties other than interaction with P-glycoprotein. Antagonists of P-glycoprotein would be an important adjunct to treatment of cancer with chemotherapeutic agents.

As an example of antagonist peptides for the mammalian energy-dependent transporters, an antagonist peptide for P-glycoprotein may be selected from the relevant TM domain amino acid sequences shown in Table 4 or may be an effective fragment or analogue of any of these sequences.

Antagonist peptides based on the amino acid sequence of a TM domain of P-glycoprotein provide therapeutic agents for combatting multidrug resistance in tumour cells, rendering them more susceptible to chemotherapeutic agents.

(c) Ion Channel Antagonists

In accordance with a further embodiment of the invention, ion channel antagonist peptides and a method for regulating or inhibiting the activity of a selected ion channel antagonist are provided.

An example of an ion channel is the γ-aminobutyric acid (GABA)-A receptor-chloride ion channel complex, which belongs to the ligand-gated receptor superfamily, which also includes the 5HT3 serotonin receptor, the nicotinic acetylcholine receptor and the metabotropic glutamate receptor.

The GABA-A receptor-chloride ion channel is believed to be a complex of five membrane-spanning protein

subunits forming a heterooligomer. The subunits belong to α, β, γ, δ or ρ class. Each subunit has an N-terminus, four putative hydrophobic membrane-spanning domains and a C-terminus, linked by extracellular and intracellular loops (Schofield et al., (1987); Bernard, E.A., 1995).

For example, an antagonist peptide for the GABA-A receptor may be selected from the following transmembrane amino acid sequences of the human α1-subunit:

TM1 YFVIQTYLPCIMTVILSQVSFW (Sequence ID NO:33);
TM2 VPARTVFGVTTVLTMTTLSISA (Sequence ID NO:34);
TM3 MDWFIAVCYAFVFSALIEFATV (Sequence ID NO:35);
TM4 LSRIAFPLLFGIFNLVYWATYL (Sequence ID NO:36)

GABA is the principal inhibitory neurotransmitter in the vertebrate brain which mediates its actions (neuronal inhibition) by binding to the integral membrane protein, the GABA-A receptor. GABA-A receptors form a fast-acting ligand-gated chloride ion-selective channel, that upon activation by agonist, results in the hyperpolarization of the neuron.

GABA-A receptor channels mediate the major inhibitory synaptic events in the brain and are involved in the regulation of anxiety, vigilance, memory, epileptogenic activity and muscle tension. GABA-A receptor subtypes have been identified in hippocampus (Pyramidal and interneurons), olfactory bulbs (Mitral and Granule cells), thalamus (relay neurons and Reticular nucleus), and in the cerebellum (Purkinje and Granule cells).

A number of drugs which have their effect on the brain act by binding to the GABA agonist site or receptor channel; these include benzodiazepines which are anxiolytic, barbiturates which are anti-convulsant, b-carbolines which are anxiogenic and picrotoxin which has convulsant effects. GABA antagonist peptides as described

herein may be used as therapeutics in similar disorders.

4. TRANSPORTER ANTAGONISTS

The family of transporter proteins are glycoproteins with twelve putative membrane-spanning domains which mediate sodium- and chloride-dependent re-uptake of neurotransmitter. The neurotransmitter transporter proteins provide a re-uptake mechanism for neurotransmitters, thereby inactivating released transmitter. This is the most important mechanism for terminating synaptic transmission of endogenous ligands such as catecholamines.

For example, for the dopamine transporter, an antagonist peptide may be selected from the following transmembrane amino acid sequences or may be an effective fragment or analogue of any of these sequences:

T1: FLLSVIGFAVDLANVWRFPYL (Sequence ID NO:37);
T2: GAFLVPYLLMVIAGMPLFYM (Sequence ID NO:38);
T3: GVGFTVILISLYVGFFYNVII (Sequence ID NO:39);
T4: WQLTACLVLVIVLLYFSLW (Sequence ID NO:40);
T5: VVWITZTMPYVVLTALLL (Sequence ID NO:41);
T6: VCFSLGVGFGVLIAFSSY (Sequence ID NO:42);
T7: IVTTSINSLTSFSSGFVVFSFL (Sequence ID NO:43);
T8:LPLSSAWAVVFFIMLLTGLI (Sequence ID NO:44);
T9: LFTLFIVLATFLLSLFCVT (Sequence ID NO:45);
T10: GTSILFGVLIEAIGVAWFYGV (Sequence ID NO:46);
T11: LCWKLVSPCFLLFVVVVSIV (Sequence ID NO:47);
T12: LGWVIATSSMAMVPIYAAY (Sequence ID NO:48).

The distribution of transporters is consistent with the distribution of neurotransmitters, suggesting that transporters might be expressed specifically for the neurotransmitter system. Transporter localization is chiefly in the presynaptic neuronal membrane.

There are also high affinity transporter proteins

for norepinephrine, glutamate, aspartate, GABA, glycine, taurine, proline, adenosine and serotonin 5-HTT.

Abnormalities of the transporter proteins have been linked to several neuropsychiatric disorders (Uhl et al., (1994), J. Exp. Biol. v. 196, pp. 229-236).

The dopamine transporter and other monoamine transporters are the target of major classes of antidepressant and psychostimulant drugs. The dopamine transporter is also targeted by drugs of abuse such as cocaine and amphetamine.

The antagonist peptides of the invention provide new, specific therapeutic agents useful in these dopamine transporter-related disorders as antidepressants and for the relief of drug craving and dependence.

The inventors have shown that peptide ALGWIIATS (Sequence ID NO:81), a fragment of the TM XII domain of the dopamine transporter, inhibited the cocaine-induced release of dopamine in caudate nucleus and nucleus accumbens of the live rat, as described in Example 10.

5. IMMUNE RECEPTOR SUPERFAMILY

The CNS, vascular and immune systems share highly conserved specific, cell surface antigen receptors or immune receptors necessary for intercellular recognition. Members of this superfamily of receptors have a large amino terminus, typically involved in antigen recognition, a single membrane-spanning domain and a carboxy tail. Some members of this superfamily, such as the FGF and VGF receptors, resemble the tyrosine kinase receptors. Examples of cell surface antigen receptors include members of the immunoglobulin receptor superfamily such as CD4.

(a) Immunoglobulin Superfamily

In accordance with a further embodiment of the

invention, immunoglobulin superfamily antagonist peptides and a method for regulating or inhibiting the activity of the selected antagonist are provided.

CD4 is a T cell specific surface glycoprotein which shows homology to members of the immunoglobulin superfamily. CD4 binds to nonpolymorphic regions of the major histocompatibility complex (MHC) class II molecule, thereby increasing the avidity of the T cell receptor for its ligand. CD4 interacts with at least two other T cell surface molecules known to be involved in T cell activation, the T cell receptor (TCR) and CD3 complex. Extensive evidence suggests that CD4 is capable of functioning as a signal transduction molecule important for the activation pathway

CD4 receptor antagonists will provide new immunosuppressives for the treatment of CD4+ T-cell mediated autoimmune diseases and allograft transplant rejection. The CD4 receptor has also been identified as a necessary major coreceptor for HIV entry into cells.

Antagonist peptides based on the amino acid sequence of the TM domain of the CD4 receptor, MALIVLGGVAGILLFIGLGIFF (Sequence ID NO:94), provide therapeutic agents useful for the treatment of autoimmune disease, the control of allograft rejection and the prevention or reduction of HIV infection.

The inventors have shown that peptide LIVLGGVAGLLLF (Sequence ID NO:181)based on the amino acid sequence of the TM domain of the CD4 inhibited HIV infection of PBMC cells, as described in Example 11.

(b) TNF/NGF superfamily

Physiological cell death (apoptosis) occurs when a cell within an organism dies by a mechanism orchestrated by proteins encoded by the organism's genome. The purpose of this process is to kill unwanted cells;

apoptosis occurs in three situations, namely, during development and homeostasis, as a defence mechanism and in aging.

Apoptosis inhibitors may be useful in treating ischemic conditions such as heart attacks, strokes or reperfusion injury, by blocking the apoptotic response of cells subjected to sublethal amounts of anoxia. They may also be useful in allograft rejection and in rheumatoid arthritis or other autoimmune disorders.

The Tumor Necrosis Factor receptors, TNFR1 and TNFR2, belong to the TNF receptor superfamily which binds TNF-α as a mediator of apoptosis.

For example, for the TNFr1 and TNFr2 receptor, an antagonist peptide may have the TM domain amino acid sequence VLLPLVIFFGLCLLSLLFIGLMY (Sequence ID NO:95) or ALPVGLIVGTALGLLIIGVVNCIMTOV (Sequence ID NO:96) respectively, or may be an effective fragment or analogue of such sequence.

Antagonist peptides based on the amino acid sequence of the TM domain of TNFr1 or TNRr2, provide therapeutic agents which can reduce or inhibit apoptosis.

(c) C type lectin superfamily

CD94 is a type II membrane glycoprotein, and is a member of the C-type lectin superfamily. CD94 receptors have been implicated in the regulation of Natural Killer (NK) cell function. An unexpected feature of CD94 is the essential absence of a cytoplasmic domain, implying that association with other receptors may be necessary for the function of this molecule.

(d) Cytokine receptors

CD95 is a member of the cytokine receptor superfamily. CD95 is a mediator of apoptosis and binds to the cytokine ligand FASL.

For example, for the CD95 receptor, an antagonist

peptide may have the transmembrane domain amino acid sequence LGWLCLLLLPIPLIVWV (Sequence ID NO:97) or may be an effective fragment or analogue of any of this sequence.

(e) EGF TM7 Superfamily

CD97 is a seven-transmembrane receptor belonging to the EGF TM7 superfamily. CD97 is expressed in leukocytes and leukocytes strongly positive for CD97 are concentrated at sites of inflammation, suggesting that CD97 may play a signal transduction role associated with the establishment or development of an inflammatory process.

(f) TM4 superfamily

CD9 is an integral cell surface protein belonging to the TM4 superfamily of receptors. CD9 is involved in the aggregation of platelets and may participate also in cell-cell interactions critical for correct orientation and movement of maturing myeloid cells in bone marrow.

6. ANTIGEN RECEPTOR ANTAGONISTS

A number of eukaryotic cell types have membrane-associated antigen receptors which are integral membrane proteins.

Exemplary of the group is the antigen receptor occurring on many mammalian cells which recognises the T cell antigen and provides the molecular basis for major histocompatability complex (MHC) antigen recognition. The receptor consists of two linked glyco-peptides, one of which, the α-glycopeptide, consists of a transmembrane domain and a cytoplasmic domain.

The amino acid sequence of the human T cell antigen receptor α chain is disclosed in Yoshikai et al. (1985) and the TM domain has the amino acid sequence:

DTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS (Sequence ID NO:49). This sequence or an effective fragment or

analogue thereof provides an antagonist which will interfere with the function of the T cell antigen receptor. Such an antagonist provides a therapeutic agent useful for control of autoimmune diseases or graft-versus-host reaction.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Materials:

Animals Male Wistar rats (Charles River Breeding Laboratories, St. Constant, QC) weighing about 250-350 gm were utilized. Upon arrival, rats were housed in the animal facility for acclimatisation for 1 week.

Peptides:

Peptides were synthesized using standard solid state methodology by commercial suppliers. Peptides were stored dessicated under refrigeration. Peptides may be synthesised with a free carboxylic acid or a C terminal amide, the amide form being preferred for its improved stability. Most of the peptides described herein were prepared in amide form. Typically for assessment of receptor antagonism, peptides were prepared at a stock concentration of 5 mg peptide per 1 ml of peptide buffer. 5 mg of peptides were dissolved in 100 ml DMSO, then diluted to a volume of 1 ml with buffer (100 mM NaCl, 10 mM Tris-HCl, 2 mM EDTA), unless indicated otherwise.

The following listed peptides are examples of TM

domain peptides prepared to provide antagonists of the indicated receptors:

1. **Peptide Antagonists of G protein-coupled Receptors**

Human dopamine D2 receptor: D2-TM I: YATLLTLLIAVIVFGNVLVC; D2-TM II: VSLAVADLLVATLVMPWVVY; D2-TM III: TLDVMMCTASILNLCAISID; D2-TM IV: RVTVMISIVWVLSFTISCPL; D2-TM V: PAFVVYSSIVSFYVPFIVTL; D2-TM VI: LAIVLGVFIICWLPFFITHI; D2-TM VII: LYSAFTWLGYVNSAVNPIIY; D2-TM VII: TWLGYVNSA

Rat α1A adrenergic receptor: α1A-AR TM VII: VFKVIFWLGYFNS; α1A-AR TM VII: VFKVIFWLGYFNSCVN;

Rat β1 adrenergic receptor: β1-AR TM VII: GYANSAFNP; β1-AR TM VII: FFNWLGYANSAFNP;

Human β2 adrenergic receptor: β2-AR TM I: LIVVGNVLVI (Sequence ID NO:98);

Human CCR5 receptor: CCR5-TM 1: LYSLVFIFGFVGN; CCR5-TM 7: MQVTETLGMT (Sequence ID NO:99);

Human CxCR4 receptor: CXCR4 TM-1: PTIYSIIFLTGIV (Sequence ID NO:100);

Human serotonin receptors: S1A-TM VII: PALLGAIIN (Sequence ID NO:101); S1B-TM VII: FHLAIFDFFTWLG (Sequence ID NO:102); S1B-TM VII: FHLAIFDFFTWLGYLNSLIN (Sequence ID NO:51); S1D-TM I: QALKISLAVVLSV (Sequence ID NO:103);

Human mu-opioid receptor: MOR-TM VII: IPETTFQTVSWH (Sequence ID NO:104);

Human angiotensin I receptor: AT1-TM VII: DTAMPITISIAY (Sequence ID NO:105); AT1-TM VII: VDTAMPITICIAYFNN (Sequence ID NO:52);

Human vasopressin 2 receptor: V2-TM VII: LMLLASLNSCTNPWIY;

Human NPY5 receptor: NPY5-TM I: YFLIGLYTFVSL (Sequence ID NO:106);

Human melanocyte stimulating hormone receptor: MSH-

TM 1: ISDGLFLSLGLVS (Sequence ID NO:107).

2. Peptide Antagonists Of Tyrosine Kinase Receptors

Human EGF-erb1 receptor: EGFR1-TM: VGALLLLLVVALG (Sequence ID NO:108);

Human EGF-erb3 receptor: EGFR3-TM: LTVIAGLVVIFMMLGG (Sequence ID NO:109); EGFR3-TM: LTVIAGLVVIF;

Human Fibroblast Growth Factor receptor: FGFr1-TM: EIIIYCTGAFLIS (Sequence ID NO:110); FGFr2-TM: VVTVILCRMKNTT (Sequence ID NO:111);

Human Vascular Endothelial Growth Factor receptor: VEGFr1-TM: SYSFQVARGMEFL (Sequence ID NO:118);

Human TrkA receptor: TrkA-TM: FASLFLSTLLLVI (Sequence ID NO:113);

3. Peptide Antagonists of Ion Channels

E. Coli ATPase Fo c subunit: GQAIAFVLFVL

E. Coli ATPase Fo b subunit: LAAIGAAIGIGILG

P-glycoprotein: VGTLAAIIHGAGL (Sequence ID NO:114)

GABA-A: GIFNLVYW (Sequence ID NO:115)

4. Peptide Antagonists of Transporters

Human dopamine transporter: DAT-TM XII: ALGWIIATS; DAT-TM XII: PDWANALGWVIIATS (Sequence ID NO:116);

5. Peptide Antagonists of Immunoreceptors

Human CD4 receptor: LIVLGGVAGLLLF

6. Peptide Antagonists of TNF receptors

Human tumor necrosis factor receptors: TNFR1-TM: TVLLPLVIFFGLSL (Sequence ID NO:117); TNFR2-TM: PVGLIVGVTALGL (Sequence ID NO:118)

7. Peptide Antagonists of Cytokine receptors

CD95-TM: WLCLLLLPIPLIVW (Sequence ID NO:119)

Example 1 - Inhibition of binding to dopamine D2 receptor

Construction of Recombinant D2 receptor encoding baculovirus: Recombinant baculovirus encoding the human D2L receptor was constructed using standard recombinant

techniques. Briefly, a cDNA clone encoding the long form of the human D2 receptor (D2L) was extracted from the pZem 3 vector with DraIII and KpnI. The resulting fragment was blunt ended and isolated by electrophoresis on soft agarose. The transfer vector pJVETLZ New was digested with NehI and blunt ended. The cDNA fragment coding for the D2L receptor was inserted into this vector by blunt-end ligation and the orientation verified by sequencing.

Transfer of recombinant baculovirus encoding the D2L receptor into the AcNPV genome was achieved by co-transfection of plasmid and wild-type viral DNA in Sf9 cells using the calcium phosphate precipitation procedure. Purification of recombinant virus was carried out as described by Vialard et al., 1990, and stocks of the purified recombinant viruses were amplified in Sf9 cells.

Construction of D2 receptor encoding pcDNA3 expression vector: The $pHD2_s$-Zem plasmid containing the entire coding sequence of the human $D2_s$ receptor was used as the template in the polymerase chain reaction (PCR) for the construction of pcDNA3 expression vectors encoding the full-length D2 receptor and truncation mutants. The D2/pcDNA3 expression vector was constructed as follows: The oligonucleotides (5'ACGCGGCCGCAGGCTGCTGTGCGGGCAGGCACGAG AGTCAGCAG TGGAGGATCTT3' and 5'GCAAGCTTGCCACCCAGTCGGTCCACCGC3') were used in the PCR reaction to generate a HindIII/NotI fragment encoding the full-length D2 receptor which was isolated by agarose gel electrophoresis and inserted into pcDNA3 at HindIII and NotI sites.

Construction of DNA plasmid encoding TM VII peptide of the D2 receptor: The D2-TM VII/pcDNA3 expression vector was constructed as follows: The oligonucleotide encoding

an initiator ATG codon, aa of TM VII of the D2 receptor, a stop TAG codon, and a XbaI site was chemically synthesized (5'ATGACGTGGCTGGGCTATGTCAACAGCGCCTGATCT AGA3'). This was annealed to a complementary oligonucleotide to produce double-stranded oligonucleotides which were digested with XbaI. The pcDNA3 vector was digested with HindIII and the ends filled with T4 DNA polymerase. The linearized pcDNA3 vector was digested with XbaI, and the double-stranded oligonucleotide subcloned into the pcDNA3 vector by blunt and sticky-end ligation. The sequence and insertion of the synthetic oligonucleotides into the vector at the appropriate site and orientation was verified by sequencing from both ends. A single oligonucleotide insert repeat was found.

Sf9 Cell Culture: Sf9 cells were grown in monolayer or suspension culture essentially as described by Summers and Smith (1987) in supplemented Grace's insect media at 27 °C. Pluronic F-68, a cell protective agent, was added to improve cell viability in suspension culture since Sf9 cells are very sensitive to mechanical shear. Cells at a density of 1-2 x 10^6/ml were infected with the recombinant virus at a multiplicity of infection of 2-5 and harvested at 24 or 48 h post-infection. Typically, viral infections were performed with a final cell density of 2 x 10^6 cells/ml. Cell viability at these times were ~90% (trypan blue-negative) as determined by trypan blue staining. To minimize variations in recombinant baculovirus infection efficiencies among batches of cultures, the same recombinant virus stock was used in all experiments for a study.

COS and CHO cell culture and DNA transfection: COS-7 monkey kidney cells (American Type Culture Collection) were maintained as monolayer cultures at 37 C in

Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and Penicillin and Streptomycin. CHO cells were cultured in Ham's F-12 media containing 10% fetal bovine serum. COS and CHO cells were transiently transfected with recombinant pcDNA3 expression vectors (8 µg) by calcium phosphate precipitation methodology as described by the manufacturer (BRL, Life Technologies Inc.). In receptor co-expression studies, D2/pcDNA3 (8 µg) was co-transfected with D2 TM-VII/pcDNA3 (8 µg) or pcDNA vector (8 µg) into COS-7 cells as described above.

Preparation of Cell Membrane Fractions: The preparation of membranes was done at 4°C. Cells were centrifuged at 100xg for 7 min. and pelleted. Cells were then washed twice with PBS and centrifuged at 100xg for 7 min (X2) and resuspended in 10 ml of buffer A: 5 mM Tris-HCl, 2 mM EDTA buffer containing the protease inhibitors: 10mg/ml benzamidine, 5 mg/ml leupeptin, and 5 mg/ml soybean trypsin inhibitor (pH 7.4 at 4°C). The cell suspension was then sonicated, two bursts at maximum setting for 15 seconds and homogenates were centrifuged at 100xg for 7 min. to pellet unbroken cells and nuclei, and supernatant was collected. The resulting pellet was homogenized a second time in 10 ml of buffer A, centrifuged as described above and supernatant fractions saved. The pooled S1 supernatant was centrifuged at high speed (27,000 xg for 20 min), washed once with buffer A, centrifuged again at high speed and resuspended in buffer A, and stored at -80 C or resuspended in buffer B: 75 mM Tris-HCl, 12.5 mM $MgCl_2$, 2 mM EDTA, pH 7.4 and assayed immediately for adenylyl cyclase activity. Pelleted membranes (P2 membranes) were resuspended in buffer A and stored at -70° C or resuspended in the appropriate buffers for immediate use in various assays.

Protein Determination: Protein content was determined by

the method of Bradford (BioRad). A standard protein concentration curve was made with bovine serum albumin (BSA). Protein concentration in the test sample was determined from the standard curve which was a plot of absorbance at 595 nm measured using a Hitachi model U-2000 spectrophotometer against concentration.

Solubilization And Immunoprecipitation Of Receptors: Membranes were prepared by sonication in buffer A as described above. The pellet was resuspended and stirred at 4°C for 2 h in 2 ml of freshly prepared solubilization buffer consisting of 100 mM NaCl, 10 mM Tris-HCl pH 7.4, 2% digitonin, and 5 mM EDTA with protease inhibitors. The homogenate was centrifuged at 27000xg for 20 min and the solubilized fraction was washed and concentrated in Centriprep 30 four times with 10 ml cold buffer C: 100 mM NaCl, 10 mM Tris-HCl pH 7.4 with protease inhibitors. The washed fraction was precleared with 1/20 normal rabbit serum and protein A-Sepharose beads for 2 h on ice. Solubilized receptors were immunoprecipitated with the mouse monoclonal 9E10 antibody (D1 receptor) or rabbit polyclonal (D2 receptor) at a 1/37 dilution in buffer C for 2 h on ice, and agitated gently overnight at 4°C with 1/40 dilution of agarose fixed goat anti-primary IgG. The immunoprecipitate was washed 6 times with 5 volumes cold buffer C for 20 min, solubilized in SDS sample buffer, sonicated and electrophoresed on SDS-PAGE as described above.

Soluble Receptor Binding: The amount of solubilized and immunoprecipitated receptor was determined by radioligand binding. D2 receptor density was estimated by incubating in the presence of saturating concentrations (~2000 pM) of the benzamide antagonist [^{3}H]YM-01951-2 *or butyrophenone antagonist [^{3}H]spiperone* for 2 h at 22°C in a total volume of 1 ml binding buffer: 100 mM NaCl, 10

mM Tris-HCl, 0.05% digitonin, 2mM EDTA with protease inhibitors, pH 7.4. Nonspecific binding was defined by binding not displaced by 1 mM (+)butaclamol. Following the incubation period, the binding preparation was loaded onto a Sephadex G-50 column (Pharmacia) and ligand bound receptor was separated from free ligand by elution. The eluate was counted in a scintillation counter for determination of the amount (pM) of receptor.

SDS-PAGE Electrophoresis: Sodium dodecyl sulphate 10-12% polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli, 1970. Immunoprecipitated membranes were solubilized in SDS sample buffer consisting of 50 mM Tris-HCl pH 6.5, 10% SDS, 10% glycerol, and 0.003% bromophenol blue with or without 10% 2-mercaptoethanol. Molecular mass (Da) of receptors was determined graphically by plotting the log molecular weight of known protein standards versus the RF (relative migration) of these proteins. The apparent molecular mass of proteins was estimated by determining the RF (from the centre of the band) and interpolating this value onto the standard curve.

Immunoblot Analysis: Membranes from cells infected with recombinant virus or with wild-type baculovirus were prepared and subjected to SDS-PAGE electrophoresis as described above and blotted on to nitrocellulose. The blots were washed in TBS for 10 min, blocked with 3% skim milk powder in TBS buffer for 30 min, washed for 10 min and incubated for 1 h at 22°C with the mouse monoclonal antibody (9E10) directed against the c-myc epitope of the c-myc-D1 receptor, or with the polyclonal antibody against a 120 amino acid sequence (nt 661-1020) in the third intracellular loop of the human D2 receptor. Primary antibodies were diluted 1/1000 in TBS containing 1% skim milk powder. Blots were then treated with 0.05%

Tween 20 in TBS for 30 min and binding of the primary antibody was detected after incubation for 1 h at 22 C with goat anti-mouse or goat anti-rabbit IgG alkaline phosphatase conjugate diluted 1/1000 in TBS containing 1% skim milk powder. Blots were then rinsed in 150 mM NaCl, 50 mM Tris-HCl pH 7.5 before developing with BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue terazolium) substrate.

Preparation of human caudate tissue: Human caudate tissue was obtained from the Canadian Brain Tissue Bank and cell membranes were isolated and receptors solubilised and immunoprecipitated as described above.

Radioligand binding studies on P2 membranes: P2 membranes were prepared as described above. Saturation binding experiments were performed with ~25 µg P2 membrane protein with increasing concentrations of [^{3}H]spiperone (10-2000 pM, final concentration) in duplicate determinations in 50 mM Tris-HCl, 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl buffer with protease inhibitors in 1 ml final volume. Competition experiments were done by preincubating P2 membranes with various amounts of peptide for 1/2 h followed by addition of [^{3}H]spiperone at a concentration equivalent to its K_D and binding buffer to a volume of 150 ml. Nonspecific binding was defined as binding that was not displaced by 1 µM (+)-butaclamol. Bound ligand was isolated by rapid filtration and washing through a Brandel 48-well cell harvester using Whatman GF/C filters. Binding data were analysed by nonlinear least-squares regression using the computer-fitting program INPLOT version 3.0 GraphPad (San Diego).

GTP-γS binding: Receptor function was assessed by receptor-mediated, agonist-stimulated [^{35}S]GTPγS binding. The assay mix contained 0.05 ml of crude membranes, 40

ml [^{35}S]GTPγS binding buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 100 mM NaCl, 20 mM GDP, ~280 pM [^{35}S]GTPγS), 40 ml peptide antagonist (5 mg in 1 ml 10% DMSO buffer). This was preincubated for 15 min at 37°C and 20 ml agonist (1 mM, final concentration) added. Bound [^{35}S]GTPγS was separated from free by filter filtration.

In Vitro Receptor disruption: 3 pmol of isolated and solubilised receptors, as determined by soluble binding assay, were prepared in buffer A (100 mM NaCl, 10 mM Tris-HCl pH 7.4, mM EDTA and 0.5% digitonin with 5 mg/ml leupeptin, 10 mg/ml benzamidine and 5 mg/ml soybean trypsin inhibitor), and incubated with 1.6 g/l (final concentration) of peptide (unless otherwise indicated). In these experiments, 5 mg of peptide were freshly dissolved in 100 ml DMSO and 100 ml Digitonin (5% w/v solution) and 800 ml buffer A. SDS buffer (50 mM Tris-HCl, pH 6.5/12% SDS/10% 2-mercaptoethanol/10% (vol/vol) glycerol/0.003% bromophenol blue) was added to the samples to make a total assay volume of 30 ml, and incubated at 37°C for 30 min prior to SDS-PAGE and immunoblot analysis.

Receptor temperature stability studies were performed with 1.5 pmol of immunoprecipitated D2 receptors. Receptors were prepared in buffer A and incubated at 23, 37, 65, and 90°C for 30 min with SDS buffer at a final volume of 30 ml, and subjected to SDS-PAGE and immunoblot analysis.

Receptor pH-stability experiments were performed with 1.5 pmol of immunoprecipitated D2 receptors prepared in buffer A. Receptors were treated with H_2O, or 0.1 N tartaric acid (final concentration), or 0.1 N HCL (final concentration), or 0.1% glacial acetic acid (final concentration). Samples were then incubated at 37°C for

30 min with SDS buffer at a final volume of 30 ml, and subjected to SDS-PAGE and immunoblot analysis.

B: Peptide-induced specific disruption of receptors: D2 receptors immunoprecipitated from membranes prepared from D2/Sf9 cells exist in the presence of sodium dodecyl sulphate (SDS) and reducing agent (2-mercaptoethanol) as ~44 kDa species at the predicted size of the D2 receptor, and as ~90 kDa species at approximately twice the molecular mass interpreted to be D2 dimers (Fig. 3A, lane 1). The dissociation of the D2 dimer to monomer was accomplished in a dose-dependent manner upon addition of the hydrophobic peptide LAIVLGVFIICWLPFFITHI, aa 375-394 of the D2 receptor, within the TM VI domain(Fig. 3B) or the peptide LYSAFTWLGYVNSAVNPIIY, aa 407-426 of the D2 receptor, within the TM VII domain(Fig. 4B). Both of these peptides, containing no strongly polar residues, had similar ability to dissociate D2 dimers immunoprecipitated from a human caudate preparation as well (Fig. 3C). A small increase in the molecular mass of receptor monomer in samples coincubated with peptide was noted, which may be attributed to the formation of a peptide-D2 receptor heterodimer.

Peptide antagonists receptor and site-specific since no disruption of D2 dimers was observed under identical experimental conditions using the hydrophilic peptide CTHPEDMKLCTVIMKSNGSF (Sequence ID NO:62), aa 244-263 of the D2 receptor C IIIA domain and peptide LSSTSPPERTRYSPIPPSHH (Sequence ID NO:63), aa 284-303) of the D2 receptor C IIIB domain or third cytoplasmic loop of the D2 receptor, or a hydrophobic peptide (aa 276-296) corresponding to a portion of the TM-VI region of the β2-adrenergic receptor (β2-AR), or two peptides derived from the carboxyl tail of the D1 receptor (aa 369-383 and aa 416-431) (Fig. 3D). In addition, no dissociation of

immunoprecipitated human dopamine D1 and serotonin 5-HT1B receptor dimers was observed with the D2-TM VII peptide fragment (Fig. 3E). These results indicate that synthetic peptides derived from hydrophobic putative transmembrane domains of receptor proteins can interact specifically to disrupt receptors as noncompetitive antagonists.

C: Physicochemical disruption of D2 receptor dimers: Consistent with the model that D2 receptors exist as dimers, D2 dimers dissociate as a function of increasing temperature (Figure 3F) or in the presence of acid (approximate pH 3.0) (Figure 3G).

D: TM peptide disruption of D2 receptor ligand binding: Peptides derived from the TM 5 (PAFVVYSSIVSFYVPFIVTL) and TM 7 (TWLGYVNSA) domains of the D2 receptor inhibited [^{3}H]-spiperone binding to P2 membranes prepared from D2 receptor-expressing Sf9 cells in a dose-dependent manner (Fig. 4). The inhibition was receptor subtype-specific, since TM peptides from the vasopressin V2 receptor-TM7 (LMLLASLNSCTNPWIY), TM-12 of the dopamine transporter (ALGWIIATS) and TM-1 of the GABA receptor A subunit (GIFNLVYW) had a much smaller effect on D2 receptor binding (Figure 4).

A peptide derived from the TM-7 domain of the D2 receptor (TWLGYVNSA) inhibited [^{3}H]-quinpirole binding to P2 membranes prepared from D2 receptor expressing Sf9 cells in a concentration-dependent manner (Fig. 5, right panel). A similar result was obtained by competition of quinpirole binding with the prototypic D2 receptor antagonist spiperone (Figure 5, left panel). These data indicate *in vitro* TM peptide inhibition of D2 receptors in accordance with the invention.

E: TM peptide inhibition of D2 receptor stimulated GTP-γS binding: In control P2 membranes, dopamine mediated a

dose-dependent stimulation of [^{35}S]-GTP-γS binding (Figure 6, left panel). Pretreatment of P2 membranes with a peptide derived from the TM-7 of the D2 receptor (TWLGYVNSA) completely inhibited the dopamine-induced activation in a dose-dependent manner (Fig. 6, right panel). The inhibition was specific to D2-derived peptides, since the peptide derived from the TM domain of the GABA-A receptor subunit (GIFNLVYW) had no effect on dopamine activation at equivalent peptide concentrations, (Figure 7, right panel). The inhibition of D2 receptor stimulated GTP-γS binding by the D2 TM-7 peptide (Figure 7, right panel) was similar in response to the prototypical D2 receptor antagonist, spiperone (Fig. 7, left panel). These data indicate in vitro TM peptide disruption of D2 receptors.

F: Co-expression of the D2-TM 7 peptide with the D2 receptor in living cells results in loss of D2 receptor binding: Co-expression of the D2 TM-7 peptide TWLGYVNSA with the full-length D2 receptor in COS-7 cells resulted in a 64% loss of saturable spiperone binding (Figure 8, open circles) relative to binding by cells expressing the D2 receptor alone (Fig. 8, solid circles), with little change in spiperone affinity. These data indicate in vivo inhibition of D2 receptors by gene-delivered TM peptide.

Example 2 - Inhibition of dopamine D2 receptor function *in vitro*

Methods

Adenylyl Cyclase Activity: Adenylyl cyclase assays were conducted essentially as described (Salomon et al., 1974). The assay mix contained 0.02 ml of membrane suspension (10-25 µg of protein), 0.012 mM ATP, 0.1 mM cAMP, 0.053 mM GTP, 2.7 mM phosphoenolpyruvate, 0.2 units of pyruvate kinase, 1 unit of myokinase and 0.13 µCi of

[^{32}P]ATP in a final volume of 0.05 ml. Enzyme activities were determined in duplicate or triplicate assay tubes containing 10^{-3} - 10^{-9} M dopamine or 100 µM forskolin or 10 mM sodium fluoride and incubated at 37° C for 30 mins. For inhibitory receptors such as the D2L receptor, adenylyl cyclase activity mix contained 100 µM forskolin. Inhibition of adenylyl cyclase assays was determine following incubation at 27° C for 20 mins. Reactions were stopped by the addition of 1 ml of an ice-cold solution containing 0.4 mM ATP, 0.3 mM cAMP and [^{3}H]cAMP (25000 cpm). Antagonist inhibition of dopamine stimulated cyclase was performed with increasing concentrations of peptide in the presence of 10 µM dopamine. cAMP was isolated by sequential column chromatography using Dowex cation exchange resin and aluminum oxide. Data were analysed by computer fitted nonlinear least-squares regression.

Co-expression of the D2-TM 7 peptide with the D2 receptor in living cells results in loss of receptor-inhibited adenylyl cyclase activity: In membranes from D2 receptor-expressing CHO cells, dopamine mediated a 13% inhibition of adenylyl cyclase activity, indicated by a decreased production of cyclic AMP (Figure 9, open bar). Dopamine inhibition of adenylyl cyclase activity was descreased to 7% in membranes from cells co-expressing the D2 TM-7 peptide with full-length D2 receptors (Fig. 9, shaded bar). These data indicate *in vivo* inhibition of the D2 receptor by gene-delivered TM peptide.

Example 3- Inhibition of dopamine D2 receptor function *in vivo*

A: METHODS

Stereotaxic surgery: Male Wistar rats (~300-350 g) were anaesthetized with ketamine (66 mg/kg i.p.), acepromazine (3 mg/kg i.p.) and pentobarbital (22 mg/kg i.p.) for

chronic stereotaxic implantation. A unilateral stainless steel guide cannula (G22) was placed into the centre of the left caudate putamen (Ant. +1.5, Lat. -2.2, Vert. -5.0, Paxinos and Watson, 1982) to allow drug or vehicle injection. Alternatively, bilateral stainless steel guide cannulas (G22) were stereotaxically placed into the centre of left and right caudate putamens for both drug and vehicle injections respectively. The guide cannula was kept patent by stylets (Plastic Products Company, Roanoke, VA) terminating 0.5 mm below the guide tips which were located 2 mm above the point of injection. The rats were allowed a week postoperative recovery before experimental use.

Intracerebral injection technique. The stylets were withdrawn and injections (drug or vehicle) made into conscious rats using a 28 gauge internal cannula connected by PE-50 polyethylene tubing to a gastight Hamilton syringe. The stylets were then replaced. For unilateral operated animals, a total of 2-3 ml drug in vehicle or vehicle alone was injected into the left striatum, followed 15 min after by a subcutaneous injection of 0.25 mg/kg apomorphine. Rats with bilateral cannulas were administered drug into the left striatum and vehicle into the right striatum, followed 15 min after by a subcutaneous injection of 0.25 mg/kg apomorphine.

Behavioural scoring. The intensity of the behavioral response was assessed every 2-5 min post-injection. Asymmetry (ipsilateral to the side of peptide or vehicle injections) was scored on the 0-3 system, 15 min after subcutaneous challenge with apomorphine (0.25 mg/kg). Animals showing an ability to move in right and left directions were not categorized as circling. However, an ability to circle in one direction (asymmetric body

posture) was scored on a 0-3 response according to observations in the open field and to the lifting of the tail. The criteria which met the 0-3 scores were:
0=no asymmetry, response of animals the same as untreated rats.
1=a distinct tendency for animals to move in one direction when handled but still capable of movement in either direction.
2=spontaneous movements in one direction, a twisting of the body in this direction, exaggerated when handled, with inability to move in opposite direction.
3=a marked and intense twisting of the body, active circling movements when disturbed, the animal being unable to move in the opposite direction.

B: INHIBITION OF D2 RECEPTOR ACTIVITY

The effect of D2 receptor transmembrane peptides on D2 receptor activity was studied using the accepted animal model described by Costall et al. (1983), for screening dopamine receptor antagonists. Effect of unilateral intrastriatal injection of receptor peptides on motor behavior. Following unilateral injection of peptides (D2-TM VII or β2-AR-TM VI) or vehicle into the left striatum, animals were observed for 1 h. All treatments failed to induce circling responses.

Circling following unilateral intrastriatal injection of D2-TM VII peptide and challenge with subcutaneous apomorphine. Subcutaneous challenge with apomorphine 15 min post unilateral intrastriatal administration of the D2-TM VII, but not β adrenergic peptide or vehicle, revealed an ipsilateral asymmetric body posturing in rats achieving a behavioral response score of 3 (Fig. 10). Similar ipsilateral asymmetric body posturing in rats was observed in bilaterally cannulated rats following intrastriatal administration of

D2-TM VII and vehicle into left and right striatum respectively (Fig. 11). The results indicate that D2-TM VII peptide acts pharmacologically as a classical D2 receptor antagonist.

Example 4 - Inhibition of binding to β adrenergic receptor

A: METHODS

Recombinant β2-adrenergic receptor encoding baculoviruses: Recombinant baculovirus encoding the c-myc epitope tagged b_2-adrenergic receptor was constructed using standard recombinant techniques, as described above. Recombinant baculovirus encoding a histidine-tagged TM-7 domain of the β_2-adrenergic receptor was constructed using the Bac-to-Bac system according to manufacturers' instructions. Briefly a complementary oligonucleotide encoding the TM-7 domain of the β_2-adrenergic receptor was ligated and subcloned into the multiple cloning site of the pFASTBAC His expression vector by standard recombinant techniques. The orientation of the cDNA was verified by sequencing. Transfer of the pFASTBAC encoding the His-tagged TM-7 of the β2-adrenergic receptor into the AcNPV genome and purification of recombinant virus was carried out as recommended by the manufacturer (BRL Life Technologies).

Cell culture and preparation of membrane fractions and solubilization of receptors: Sf9 cell culture, preparation of membrane fractions and solubilization of receptors were as described in Example 1.

Ni-NTA purification of histidine-tagged proteins: Sf9 cells co-expressing histidine-tagged β2-AR Tm7 peptide and full-length c-myc tagged β2-AR were disrupted under native conditions. The cell lysate was passed by gravity-flow over a Ni-NTA resin to purify histidine-

tagged proteins. Histidine-tagged proteins generated in Sf9 cells were eluted from the resin according to the BAC-to-BAC kit instructions (BRL, Life Technologies).

SDS-PAGE and Immunoblot Analysis: were carried out as in Example 1. Immunoreactive histidine-tagged β_2-AR TM 7 peptide was revealed with a rabbit polyclonal antibody raised against the 6XHIS sequence (Santa Cruz Biotech. Inc.). Immunoreactive c-myc epitope-tagged β_2-AR was detected with mouse monoclonal 9E10 antibody, raised against a peptide corresponding to amino acids 408-439 of the human c-myc protein (Santa Cruz Biotech. Inc.).

Soluble β2-adrenergic receptor binding: β2-adrenergic receptor binding was estimated by incubating solubilized membranes from β2-adrenergic receptor expressing Sf9 cells (75 ml) in the presence of saturating concentrations of ^{3}H-alprenolol (15 ml) for 2 hrs at 22 °C in a total volume of 150 ml soluble binding buffer (described above). Competition experiments were done by preincubating soluble β2-adrenergic receptors with peptide (40 ml of a 5 mg /ml peptide stock made in 10% DMSO buffer) for 1/2 h followed by addition of [^{3}H]alprenolol and binding buffer to a volume of 150 ml. Non-specific binding was defined as binding not displaced by 1 mM pindolol. Bound receptor was separated from free ligand by elution from Sephadex G-50 columns and binding activity determined by scintillation counting.

B: Immunological detection of a histidine-tagged β2-TM 7 peptide - c-myc epitope tagged β2 receptor heterodimer: To ascertain that peptides based on TM domains of a receptor interacted with the target receptor, a histidine-tagged TM-7 peptide of the β_2-adrenergic receptor was co-expressed with a full-length c-myc-

epitope tagged β_2-adrenergic receptor in Sf9 cells. A histidine-tagged β2-TM 7 peptide - c-myc epitope tagged β_2 receptor heterodimer should be detectable on immunoblots of Ni-NTA purified receptors. An antibody against the His tag of the TM-7 peptide revealed on immunoblot a species at the expected molecular mass (~50 kDa) of a heterodimer (Figure 12, right panel). In the identical preparation, the same species was detected as an immunoblot with the 9E10 antibody, (Figure 12, left panel), demonstrating interaction of the transmembrane-derived peptides with the full-length receptor. These data indicate *in vitro* TM peptide inhibition of adrenergic receptors.

C: Inhibition of β_2-adrenergic receptor binding by subtype selective peptides: [^{3}H]-alprenolol binding to the β_2-adrenergic receptor was inhibited by a peptide derived from the TM-1 domain of the β_2-adrenergic receptor (β2-AR TM I: LLIVVGNVLVI) (Fig. 13A). Similar results were obtained for the prototypical β_2-adrenergic receptor antagonist pindolol (Fig. 13B). A peptide derived from the TM-7 of the β_1-adrenergic receptor (β1-AR TM VII:GYANSAFNP), two peptides derived from the TM domain of the ATP-ase subunit (*E. Coli* ATPase Fo b subunit: GQAIAFVLFVL; *E. Coli* ATPase Fo c subunit: LAAIGAAIGIGILG), and a peptide derived from the TM 1 domain of the CCR5 receptor (LYSLVFIFGFVGN) had a negligible effect on [^{3}H]-alprenolol binding (Fig. 13A). These data indicate highly specific inhibition of an adrenergic receptor by peptides derived from the transmembrane domain of the receptor, but not by peptides derived from the transmembrane domain of the closely related β1 adrenergic receptor.

Example 5 - Inhibition of α adrenergic and β adrenergic

receptor activity *in vivo*

A: METHODS

Animals Male Wistar rats (Charles River Breeding Laboratories, St. Constant, QC) weighing about 250-350 gm were utilized. Upon arrival, rats were housed in the animal facility for acclimatisation for 1 week.

Surgery Prior to the experiments, the rats were fasted overnight but provided with water *ad libitum*. Under halothane anesthesia, the left femoral or jugular vein was exposed, cleaned, clamped and cannulated with polyethylene tubing (Tygon; PE 10-20) for intravenous injections of drugs. The right carotid artery was then exposed adjacent to the trachea and cannulated for blood pressure recording (PE 10-20 tubing). The cannulae were tunneled subcutaneously to the midback of the animal where they were brought out onto the skin surface and capped with rubber injection ports. All catheters were filled with a solution of heparin (10 units/ml) and were flushed periodically with the same solution to prevent clotting.

Cardiovascular measurements Rats were allowed to wake up and recover for 3 hrs prior to cardiovascular measurements. A transducer was connected to the carotid arterial catheter, and blood pressure was recorded on a polygraph. Animals were given atropine 1 mg/Kg subcutaneously 2 to 3 hours before measurement of adrenergic responses to antagonise cholinergic tone.

Measurement of adrenergic receptor responses All drugs were injected intravenously (femoral or jugular vein) in bolus volumes of 0.1 - 0.2 ml and flushed in with 0.2 ml of saline. Blood pressure was recorded continuously by polygraph.

B: Inhibition of β1-adrenergic receptor activity

The effect of adrenergic receptor antagonist

peptides was examined using an accepted animal model for assessing cardiovascular drugs.

After a period of recording baseline cardiac function, in an awake and freely-mobile rat, (480 beats/min, with systolic/diastolic blood pressure of 160/110 min Hg, as seen in Figure 14A), the rat was given 1 mg/Kg of the β1-adrenergic receptor agonist, isoproterenol, by rapid IV bolus.

This produced a marked increase in spontaneous cardiac activity, with a rise to 600 beats/min and an accompanying reduction in blood pressure to 140/70 (Figure 14B).

After an interval to allow baseline parameters to be reestablished, 100 ml of 5 mg/ml peptide FFNWLGYANSAFNP, a fragment of the β1-adrenergic receptor TM VII peptide, (β1-TM7) was administered intravenously.

As seen in Figure 14C, there was a marked reduction in spontaneous cardiac activity to 240 beats/min with a selective drop in diastolic blood pressure to give 160/60, indicative of β1-adrenergic receptor antagonism.

After baseline parameters were again reestablished, a further 1 mg/Kg isoproterenol was administered. The response to isoproterenol was attenuated, as seen in Figure 14D, showing lasting β1-adrenergic receptor antagonism induced by the TM VII peptide fragment

Figures 14E to 14H show the results of a control experiment. Baseline cardiac parameters were 240 beats/min and blood pressure 130-125/80 (Fig. 14E). Fig. 14F shows the response to 1 mg/Kg isoproterenol, resulting in reduced baseline blood pressure 125/60 characteristic of β1-adrenergic receptor activation. Administration of peptide vehicle (Buffer A + 10% DMSO) slightly reduced blood pressure to 140/50 but did not

change spontaneous cardiac activity (Fig. 14G).

After baseline parameters were reestablished, exposure of the animal to 1 mg/Kg isoproterenol showed characteristic heart beat and blood pressure changes (Fig. 14H) with no attenuation of the drug's initial effect.

C: Inhibition of α1A-adrenergic receptor activity

After a period of recording baseline cardiac function in an awake and freely-mobile rat (360 beats/min with systolic/diastolic blood pressure of 130/90, as seen in Figure 15A), the rat was given 5 mg/Kg phenylephrine by rapid IV bolus. Heart rate was reduced to 240 beats/min, with blood pressure rising to 180/140, reflecting a drug-induced vasoconstriction effect (Fig. 15B).

After an interval to allow baseline parameters to be reestablished, 100 ml of 5 mg/ml peptide VFKVIFWLGYFNSCVN, a fragment of the α1A-adrenergic receptor TM VII peptide was administered intravenously. As seen in Figure 15C, heart rate was reduced to 240 beats/min before recovery to 420 beats/min, during which time there was a significant, transient drop in blood pressure to 130-120/60.

After baseline parameters were again reestablished, a further 5 mg/Kg phenylephrine was administered. The response to phenylephrine was delayed and attenuated indicating lasting α1A-adrenergic receptor antagonism induced by the TM VII peptide fragment (Figure 15D). Figures 15E-15G show the results of a control experiment in the same rat. Administration of saline resulted in an unexpected transient increase in blood pressure from 150/100 to 180/100, with no change in heart rate, before stabilising to baseline values (Figure 15E).

Administration of peptide vehicle had no significant

effect on heart rate, but lowered blood pressure marginally from 150/100 to 140/80 (Fig. 15G). Subsequent administration of 5 mg/Kg phenylephrine showed no diminution of effect and reduced heart rate to 240 beats/min with blood pressure rising to 180/140 (Fig. 15F).

As a comparison, the effect of prasozin, a classical α1A-adrenergic receptor antagonist, was examined (Figures 15H to 15K).

Prasozin (1 mg/Kg by rapid IV bolus) had an effect on heart rate and blood pressure (Figure 15J) similar to that of the antagonist TM VII peptide fragment and also caused attenuation of the response to a subsequent administration of the agonist phenylephrine (Fig. 15K).

Example 6 - Inhibition of vasopressin V2 receptor *in vivo*

A: METHODS

Rats and surgery: Sprague Dawley rats were obtained from Charles River (220-230 g). Rats were acclimizatized for 48 h prior to experiments. Rats were anesthesized with halothane and nephrectomized (right kidney) and placed back into the vivarium for seven days. Following this post-operative recovery time, rats weighed 250-270 g. Rats were then anesthesized with sodium pentobarbital at a dose of 36 mg/ kg (i.p.) and maintained under anesthesia for the duration of the experiment with 3 mg pentobarbital doses as necessary.

A catheter was placed in the right carotid artery (PE 50) for measuring blood pressure, one in the left jugular vein (PE 60) for injection of vehicle, peptide, or drugs and one in the left ureter (PE50) for collecting urine.

Following surgery, rats were stabilized for 45 min. Mean arterial pressure was measured for 15 min (baseline), at which time various drugs were injected via

jugular vein and mean arterial pressure (MAP) and urine output recorded.

V2 antagonist Peptide: V2 receptor peptide antagonism and its effects on MAP and urine output was assessed by single bolus dose of 1 mg peptide (V2-TM VII: LMLLASLNSCTNPWIY) in 200 ml vehicle (water with 10% DMSO and protease inhibitors).

Bolus injection of the V2-TM VII peptide (V2 AT) resulted in a marked increase in urine output, from baseline urine flow of 1100 ml/15 min to 1900 ml/15 min, which was sustained for 45 min. (Figure 16). In comparison, injection of the vehicle alone had only a small effect on increasing urine output (Figure 16). These data show that receptor-subtype specific TM peptides act as antagonists of V2 receptors.

Example 7 - Inhibition of HIV infectivity by antagonists of the CCR5 receptor

A: METHODS

CCR5 Peptide Antagonist Treatments: 10 mg of peptide (CCR5-TM 1: LYSLVFIFGFVGN-NH2) was dissolved in 500 ml of DMSO, and 50 ml was used to pretreat 1 ml of cultured cells (4 X 10x6 cells/well) for ½ h. Peptide concentration at this stage was 0.5 mg/ml. Peptide concentration was then diluted in half upon addition of virus for 2 h, in a total assay volume of 2 ml.

HIV-1 Infectivity Assay As Assessed By HIV-RT Activity: HIV-1 Infectivity Assay was performed according to methods described by Mark A. Wainberg (Soudeyns et al., Antimicrobial Agents and Chemotherapy 35, 1386-1390, 1991).

HIV-IIIB isolate of HIV-1 was employed because of its high infectiousness. Briefly, human PBMC cells were infected at a multiplicity of infection of 0.1. To determine the levels of inhibition of HIV-1 adsorption,

by CCR5-TM1, PBMC cells were pretreated for 1/2 h with the peptide, exposed to the virus for 2 h and viral infection was assessed 7 days post-infection by estimation of viral cDNA by RT-PCR levels in culture

HIV-1 Infectivity Assay As Assessed By Viral P24-antigen Production The CCR5-TM peptide antagonist (CCR5-TM 1: LYSLVFIFGFVGN-NH2) was tested for anti-M tropic virus activity as described in Brennan et al., (1995), Antiviral Research, **26**,173 and Taylor et al., (1995), Antiviral Research, **28**, 159.

B: Peptide inhibition of cellular adsorption and uptake of HIV as assessed by RT-activity: In human PBMC cells pretreated with CCR5 TM1, the HIV reverse transcriptase activity was 26m757.5 ± 4051 cpm (mean of triplicate wells), compared to 128,792 ± 11996 cpm (mean of triplicate wells) for non-peptide treated cells. These data indicate 79% inhibition of HIV infection by the CCR5 peptide antagonist (Fig. 17A).

C: Peptide inhibition of HIV cellular adsorption and uptake as assessed by P24-antigen production: In PBMC cells pretreated with CCR5-TM1, the HIV P24 level was 4730 pg/ml compared to 20960 pg/ml for non-peptide treated cells. These data indicate 77% inhibition of HIV infection by the CCR5 peptide antagonist (Fig. 17B). These data show that CCR5-TM peptide acted as an antagonist of the CCR5 receptor and inhibited HIV infection.

Example 8 - Inhibition of EGF-mediated tyrosine kinase activity

A: METHODS

Cell Culture: The human A431 cell line (ATCC CRL 1555) was established from an epidermoid carcinoma of a 85 year old female patient. It expresses an extremely high number of EGF receptors on its cell surface (3 x10E6/cell), due,

at least in part, to the amplification of EGF receptor DNA sequences (30-fold).

EGF receptor tyrosine kinase enzyme assay: The Biotrak* assay system and kit by Amersham Life Science was used. In brief, the system is designed to detect epidermal growth factor receptor tyrosine kinase enzyme activity in solubilized tissues and cells. Enzyme present in the samples will catalyze the transfer of the g-phosphate of adenosine-5^t-triphosphate to the tyrosine group on a peptide which is specific for EGFr tyrosine kinase domain. Specific detection of the enzyme is further assured by using epidermal growth factor to activate the EGF receptor tyrosine kinase enzyme activity. Thus EGF dependent tyrosine kinase activity may be determined from the difference between the enzyme activity in the presence or absence of added epidermal growth factor. The assay is performed at pH 7 .4 in Hepes buffer with $MgCl_2$ as the essential metal ion. The assay will give linear incorporation of phosphorus-32 into substrate peptide corresponding to at least 20% of ATP incorporated, providing samples are suitably diluted. Phosphorylated peptide is separated on binding paper. After washing the paper, the extent of phosphorylation may be detected by scintillation counting.

A solubilized membrane preparation was prepared from A431 cells maintained in culture as a monolayer, and preincubated for 30 min at 30 °C with various concentrations of a peptide derived from the TM domain of the EGF-erb3 receptor (EGFR3-TM: LTVIAGLVVIF) or with peptides derived from TM domains of other receptors (D2-TM VII-TWLGYVNSA; GABA-A: GIFNLVYW) prior to tyrosine kinase assay.

B: EGF-erb3 receptor TM peptide inhibited EGF mediated tyrosine kinase activity. The EGFr peptide antagonist

(LTVIAGLVVIF) inhibited EGF mediated tyrosine kinase activity in a dose-dependent manner, in comparison to a peptide derived from GABA TM domain (GIFNLVYW) (Fig. 18). Peptide D2-TM VII (TWLGYVNSA) had a similar lack of effect on EGF mediated tyrosine kinase activity (data not shown).

Example 9 - Inhibition of bacterial growth

A: METHODS

Peptide treatments: A peptide derived from *E. Coli* ATPase Fo b subunit, GQAIAFVLFVL and the peptide derived from *E. Coli* ATPase Fo c subunit, LAAIGAAIGIGILG were tested for antimicrobial activity in comparison to a peptide derived from the GABA-A ion channel (GIFNLVYW), and a peptide derived from the D2 dopamine receptor (D2-TM VII-TWLGYVNSA). 5 mg of each peptide was dissolved in DMSO and diluted with water (DMSO concentration was 10% of the final volume). When tested alone, 50 ml of peptide solution was used for each 500 ml culture. When tested in combination, 25 ml of each peptide solution was used for each 500 ml culture.

Gram negative E. Coli culture and antimicrobial assay: *E. coli* strain LE392 was grown in LB medium to a density of O.D.600 between .8-1.0. A 10:1 dilution was made for a final volume of 500 ml. 50 ml of test peptide (5 mg/ml stock concentration) was added to the culture (0.5 ug/ul, final peptide concentration) which was then grown at 37 C for 2 h. 50 ml of the culture was then plated on to LB plates at 10^{-6} dilution. Plates were then incubated overnight and colonies counted the next morning. The remaining culture was stored at 4 °C overnight. 50 ml of this culture was then plated the following day on to LB plates at 10^{-6} dilution. Plates were incubated overnight and colonies counted next morning.

B: Antimicrobial activity of TM peptides derived from

the Fo c and b subunits of the Gram negative *E. coli* bacteria. Peptides derived from the Fo b and Fo c subunits, used either singly or in combination, suppressed the growth of E. coli. In one experiment, when *E. coli* cultures were treated with a combination of the peptides derived from the Fo b and c subunits, there were 9 colonies compared to 31 for vehicle treated cells, and 29 for cells treated with a peptide derived from the GABA-A channel subunit (Fig. 19). In a further experiment, peptides derived from the Fo b and c subunits used in combination resulted in total killing of bacteria.

These data show that transmembrane peptides derived from bacterial transporter proteins act as potent antimicrobial agents.

Example 10 - Inhibition of cocaine-mediated dopamine release

A: METHOD

In vivo microdialysis to measure DA transporter function:

Male Wistar rats (Charles River Canada; 200-250 g) were individually caged in environmental rooms under constant conditions of temperature, humidity and 12 hr light-dark cycles. Food was available ad libitum. Animals were handled and weighed daily to minimize stress on experimental days. Rats were implanted with intracerebral guide cannulae (Plastic Products Company, Roanoke, VA, U.S.A.) for the microdialysis probes (Carnegie Medicin, Sweden) under anaesthesia with ketamine (66 mg/kg) and pentobarbital (22 mg/kg). The cannula was inserted into the medial nucleus accumbens (coordinates relative to bregma: A +1.5, L +1.3, V +7.6, Paxinos and Watson, 1982) or caudate nucleus (coordinates relative to bregma: A +1.6, L +2.3, V -3.8) for microdialysis sampling. The animals were allowed to recover for a minimum of 3 days before sampling studies

were conducted. At the end of the experiments, probe placement was verified anatomically in each case by sectioning the frozen brain using a microtome-cryostat. Results reported are only from those animals in which the probe was confirmed to be in the medial part of the nucleus accumbens or caudate nucleus histologically.

On the day of the experiment, the steel insert from the guide cannula was replaced by the dialysis probe (2 mm, CMA/12, Carnegie Medicin, Sweden) and perfused with artificial CSF (NaCl 145 mM, KCl 2.7 mM, $CaCl_2.2H_2O$ 1.2 mM, $MgCl_2$ 1.0 mM, Na_2HPO_4 2 mM, ascorbic acid 0.2 mM, pH 7.4) at a rate of 1 ml/min. Perfusion in the awake, unrestrained and mobile animals was continued for 3-4 hours until the basal efflux of dopamine and its metabolites were stable for 3 consecutive measurements. Dialysate was collected over 30 min periods and injected directly into a HPLC system equipped with a Biophase ODS 5 mm, 4.6 x 250 mm column, Waters 590 pump with U6K injector and ESA 5100A electrochemical detector with Model 5011 analytical cell for measurement of DA, DOPAC and HVA. The mobile phase consisted of 50 mM sodium phosphate monobasic, 0.5 mM EDTA, 1.8 mM sodium octyl sulfonate, 14 % methanol, with pH adjusted to 3.50 with phosphoric acid. Sensitivity of dopamine detection was 2 pg. The percentage recovery of dopamine through the dialysis cannula was calculated each time.

B: Inhibition of cocaine-mediated dopamine release

An antagonist peptide for the dopamine transporter (DAT) was made based on the amino acid sequence of TM-12 (DAT-TM XII:ALGWIIATS). In animals given cocaine (5 mg/kg i.p.) there was a characteristic rapid release of dopamine measured by microdialysis sampling in the striatum, as shown in Fig. 20 (solid circles).

Pretreatment with the antagonist peptide for the dopamine transporter (70 mg given intracerebroventricularly by slow infusion in 7 ml of buffered saline 12 min before injection of cocaine) resulted in the complete abolition of the dopamine release mediated by cocaine (Fig. 20, open circles). Intracerebroventricular injection of the dopamine transporter antagonist peptide alone in doses of 70 mg or 98mg had no effect on dopamine release in striatum (Fig. 21).

In animals given cocaine (5 mg/kg i.p.), there was a characteristic rapid release of dopamine measured by microdialysis sampling in the nucleus accumbens, as shown in Fig. 22, solid circles. Pretreatment with the antagonist peptide for the dopamine transporter (150 mg given intracerebroventricularly by slow infusion in 7 ml of buffered saline 12 min before injection of cocaine) resulted in marked attenuation of the dopamine release mediated by cocaine (Fig. 22, open circles).

These data show that transmembrane peptides derived from the dopamine transporter act are effect to counteract the effect of cocaine on the transporter.

Example 11 - Inhibition of HIV infectivity by antagonists of the CD4 receptor

A: METHODS

Peptide inhibition of HIV cellular adsorption and uptake as assessed by P24-antigen production. In cells pretreated with the CD4-TM peptide antagonist LIVLGGVAGLLLF, for 1 hour, the HIV P24 level was 14970 pg/ml compared to 20960 pg/ml for non-peptide treated cells. These data indicate 29% inhibition of HIV infection by the CD4-TM peptide antagonist (Fig. 23).

These data show that the CD4-TM peptide acted as an inhibitor of HIV infection.

The present invention is not limited to the features

of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

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TABLE 1A - G PROTEIN-COUPLED RECEPTORS

Receptor	Accession #	Receptor	Accession #
Adenosine A_1	P30542	Chemokine CXCR1	L19591*
Adenosine A_{2A}	P29274	Chemokine CXCR2	M73969*
Adenosine A_{2B}	P29275	Chemokine CXCR3	U32674*
Adenosine A_3	P33765	Chemokine CXCR4	M86739*
Adrenergic α_{1A}	P25100	Cholecystokinin CCK_A	P32238
Adrenergic α_{1B}	P35368	Cholecystokinin CCK_B	P32239
Adrenergic α_{1D}	M60654*		
Adrenergic α_{2A}	P08913	Dopamine D1	P21728
Adrenergic α_{2B}	P18089	Dopamine D2	P14416
Adrenergic α_{2C}	P18825	Dopamine D3	P35462
Adrenergic β_1	P08588	Dopamine D4	P21917
Adrenergic β_2	P07550	Dopamine D5	P21918
Adrenergic β_3	P13945		
		Endothelin ET_A	P25101
Adrenomedullin	L09249*	Endothelin ET_B	P24530
Anaphylatoxin C3a	U28488*	Follicle-stimulating hormone	P23945
Anaphylatoxin C5a	P21730		
Angiotensin AT_{1A}	X62295*	Formyl-peptide FPR1	P21462
Angiotensin AT_{1B}	Q13725	Formyl-peptide FPR2	M84562*
Angiotensin AT_2	P50052		
		Galanin	P47211
Bombesin BB_1	U37058*		
Bombesin BB_2	M57922*	Gastrin receptor	P32239
Bombesin BB_3	P32247		
		Gonadotropin-releasing hormone	P30968
Bradykinin B_1	P46663		
Bradykinin B_2	P30411		
		Histamine H_1	P35367
Calcitonin-gene related peptide 1	Q16602	Histamine H_2	P25021
		Lutropin-choriogonadotropin hormone (LH-CG)	P22888
Cannabinoid CB1	P21554		
Cannabinoid CB2	P34972		
		Lysophosphatidic acid	U76385*
Chemokine CCR1	P32246		
Chemokine CCR2	P41597		
Chemokine CCR3	P51677	Melanocortin MC1	X65634*
Chemokine CCR4	P51679	Melanocortin MC2	Q01718
Chemokine CCR5	P51681	Melanocortin MC3	P41968
		Melanocortin MC4	P32245
		Melanocortin MC5	P33032

TABLE 1B - G-PROTEIN COUPLED RECEPTORS

Receptor	Accession #	Receptor	Accession #
Melatonin ML_{1A}	P48039	Prostanoid EP_1	P34995
Melatonin ML_{1B}	P49286	Prostanoid EP_2	P43116
		Prostanoid EP_3	P43115
Muscarinic Acetylcholine M1	P11229	Prostanoid EP_4	P35408
		Prostanoid DP	D29764*
Muscarinic Acetylcholine M2	P08172	Prostanoid FP	P43088
		Prostanoid IP	P43119
Muscarinic Acetylcholine M3	P20309	Prostanoid TP	D38081*
Muscarinic Acetylcholine M4	P08173	Protease-activated 1	M62424*
		Protease-activated 2	Z35158*
Muscarinic Acetylcholine M5	M80333*		
		Purinoceptor PS_{Y1}	X73268*
		Purinoceptor $P2_{Y2}$	P41231
Neurokinin NK1 (substance P)	J05097*	Purinoceptor $P2_{Y3}$	X98283*
		Purinoceptor $P2_{Y4}$	U40223*
Neurokinin NK2 (substance K)	P21452	Purinoceptor $P2_{Y5}$	L06109*
		Purinoceptor $P2_{Y6}$	D63665*
Neurokinin NK3 (neuromedin K)	P29371	Purinoceptor $P2_{Y7}$	U41070*
Neurokinin NK4	M84605*	Rhodopsin	P08100
Neuropeptide Y Y1	P25929	Serotonin 5-HT_{1A}	P08908
Neuropeptide Y Y1-like	U58367*	Serotonin 5-HT_{1B}	P28222
Neuropeptide Y Y2	P49146	Serotonin 5-HT_{1D}	P28221
Neuropeptide Y Y4	P50391	Serotonin 5-HT_{1E}	P28566
Neuropeptide Y Y5	Q61212	Serotonin 5-HT_{1F}	P30939
		Serotonin 5-HT_{2A}	P28223
Neurotensin NTR1	P30989	Serotonin 5-HT_{2B}	P41595
Neurotensin NTR2	X97121*	Serotonin 5-HT_{2C}	P28335
		Serotonin 5-HT_4	U20907*
Opioid δ	P41143	Serotonin 5-HT_{5A}	P47898
Opioid κ	P41145	Serotonin 5-HT_{5B}	X69867*
Opioid μ	P35372	Serotonin 5-HT_6	P50406
Orphanin	P41146	Serotonin 5-HT_7	P34969
Oxytocin	P30559	Somatostatin sst_1	P30872
		Somatostatin sst_2	P30874
Platelet-activating factor	P25105	Somatostatin sst_3	P32745
		Somatostatin sst_4	P31391
		Somatostatin sst_5	P35346
		Thyrotropin-releasing hormone	M37490*

TABLE 1C - G-COUPLED PROTEIN RECEPTORS

Receptor	Accession #
Thyrotropin-stimulating hormone (TSH)	M29957*
Vasopressin V_{1A}	P37288
Vasopressin V_{1B}	P47901
Vasopressin V_2	P30518
Secretin	P47872
Calcitonin	P30988
Parathyroid hormone	P49190
Parathyroid hormone related peptide	Q03431
VIP	P32241
Glucagon	P47871
Glucagon-like peptide 1	P43220
Growth hormone releasing hormone	Q02643
Pituitary adenylate cyclase activating polypeptide	P41586
Corticotropin releasing factor	Q13324
Gastric inhibitory polypeptide	P48546
Calcium sensing	P41180

TABLE 2

DOPAMINE D2 RECEPTOR, Human

443 Amino acids

ACCESSION: P14416

FEATURES

DOMAIN	1	37	EXTRACELLULAR (POTENTIAL).
TRANSMEM	38	60	1 (POTENTIAL).
DOMAIN	61	71	CYTOPLASMIC (POTENTIAL).
TRANSMEM	72	97	2 (POTENTIAL).
DOMAIN	98	108	EXTRACELLULAR (POTENTIAL).
TRANSMEM	109	130	3 (POTENTIAL).
DOMAIN	131	151	CYTOPLASMIC (POTENTIAL).
TRANSMEM	152	174	4 (POTENTIAL).
DOMAIN	175	186	EXTRACELLULAR (POTENTIAL).
TRANSMEM	187	210	5 (POTENTIAL).
DOMAIN	211	373	CYTOPLASMIC (POTENTIAL).
TRANSMEM	374	397	6 (POTENTIAL).
DOMAIN	398	405	EXTRACELLULAR (POTENTIAL).
TRANSMEM	406	429	7 (POTENTIAL).
DOMAIN	430	443	CYTOPLASMIC (POTENTIAL).

SEQUENCE

MDPLNLSWYD DDLERQNWSR PFNGSDGKAD RPHYNYYATL LTLLIAVIVF GNVLVCMAVS
REKALQTTTN YLIVSLAVAD LLVATLVMPW VVYLEVVGEW KFSRIHCDIF VTLDVMMCTA
SILNLCAISI DRYTAVAMPM LYNTRYSSKR RVTVMISIVW VLSFTISCPL LFGLNNADQN
ECIIANPAFV VYSSIVSFYV PFIVTLLYVI KIYIVLRRRR KRVNTKRSSR AFRAHLRAPL
KGNCTHPEDM KLCTVIMKSN GSFPVNRRRV EAARRAQELE MEMLSSTSPP ERTRYSPIPP
SHHQLTLPDP SHHGLHSTPD SPAKPEKNGH AKDHPKIAKI FEIQTMPNGK TRTSLKTMSR
RKLSQQKEKK ATQMLAIVLG VFIICWLPFF ITHILNIHCD CNIPPVLYSA FTWLGYVNSA
VNPIIYTTFN IEFRKAFLKI LHC

TABLE 3

	Accession #
IMMUNOGLOBIN super family	
CD4	P01730
TNF/NGF superfamily	
TNF-α1	P19438
TNF-α2	P20333
EGF erb1	P00533
IL-1	P14778
CD95	P25445
EGF-TM7 family	
CD97	P48960
TM4 superfamily	
CD9	P21926
C Type Lectin superfamily	
CD 94	
Ion channels	
GABA-A	P14867
p-glycoprotein	P08183
bacterial ATPase	Z26850*
Tyrosine kinase	
Fgr1	P11362
Fgr2	P21802
Vgr1	P17948
VGR2	P35968
TrkA	P04692

TABLE 4A

HUMAN CC CHEMOKINE (CCR5) RECEPTOR

Transmembrane Domain 1:
LLPPLYSLVFIFGFVGNMLVILILINC
(Sequence ID NO:120)
Transmembrane Domain 2:
LLNLAISDLFFLLTVPFWAHY
(Sequence ID NO:121)
Transmembrane Domain 3:
LLTGLYFIGFFSGIFFIILLTI
(Sequence ID NO:122)
Transmembrane Domain 4:
VTFGVVTSVITWVVAVFASLPGIIF
(Sequence ID NO:123)
Transmembrane Domain 5:
VILGLVLPLLVMVICYSGIL
(Sequence ID NO:124)
Transmembrane Domain 6:
LIFTIMIVYFLFWAPYNIVLLLNTF
(Sequence ID NO:125)
Transmembrane Domain 7:
AMQVTETLGMTHCCINPIIYAFVG
(Sequence ID NO:126)

HUMAN FUSIN (CXCR4) RECEPTOR

Transmembrane Domain 1:
FLPTIYSIIFLTGIVGNGLVILVMG
(Sequence ID NO:127)
Transmembrane Domain 2:
LSVADLLPVITLPFWAVDAV
(Sequence ID NO:128)
Transmembrane Domain 3:
AVHVIYTVNLYSSVLILAFISL
(Sequence ID NO:129)
Transmembrane Domain 4:
VVYVGVWIPALLLTIPDFIFA
(Sequence ID NO:130)
Transmembrane Domain 5:
FQHIMVGLILPGIVILSCYCII
(Sequence ID NO:131)
Transmembrane Domain 6:
TVILILAFFACWLPYYIGISI
(Sequence ID NO:132)
Transmembrane Domain 7:
ITEALAFFHCCLNPILYAFLG
(Sequence ID NO:133)

HUMAN 5HT1A RECEPTOR

Transmembrane Domain 1:
QVITSLLLGTLIFCAVLGNACVVAAIAL
(Sequence ID NO:134)
Transmembrane Domain 2:
LIGSLAVTDLMVSVLVLPMAALYQVL
(Sequence ID NO:135)
Transmembrane Domain 3:
DLFIALDVLCCTSSILHLCAIAL
(Sequence ID NO:136)
Transmembrane Domain 4:
AAALISLTWLIGFLISIPPMLGW
(Sequence ID NO:137)
Transmembrane Domain 5:
DHGYTIYSTFGAFYIPLLLMLVLYG
(Sequence ID NO:138)
Transmembrane Domain 6:
TLGIIMGTFILCWLPFFIVALVL
(Sequence ID NO:139)
Transmembrane Domain 7:
LLGAIINWLGYSNSLLNPVIYAY
(Sequence ID NO:140)

HUMAN MU OPIOID RECEPTOR

Transmembrane Domain 1:
MITAITIMALYSIVCVVGLFGNFLVMYV
IV(Sequence ID NO:141)
Transmembrane Domain 2:
TATNIYIFNLALADALATSTLP
(Sequence ID NO:142)
Transmembrane Domain 3:
IVISIDYYNMFTSIFTLCTMSV
(Sequence ID NO:143)
Transmembrane Domain 4:
WILSSAIGLPVMFMATT
(Sequence ID NO:144)
Transmembrane Domain 5:
ICVFIFAFIMPVLIITVCYGLMI
(Sequence ID NO:145)
Transmembrane Domain 6:
MVLVVVAVFIVCWTPIHIYVII
(Sequence ID NO:146)
Transmembrane Domain 7:
TFQTVSWHFCIALGYTN
(Sequence ID NO:147)

HUMAN MELANOCYTE STIMULATING HORMONE (MSH) RECEPTOR

Transmembrane Domain 1:
VSISDGLFLSLGLVSLVENALVVATIA
(Sequence ID NO:148)
Transmembrane Domain 2:
MYCFICCLALSDLLVSGT NVL
(Sequence ID NO:149)
Transmembrane Domain 3:
VIDVITCSSMLSSLCFLGAIAV
(Sequence ID NO:150)

TABLE 4A (Continued)

Transmembrane Domain 4:
AVAAIWVASVVFSTLFIAYYD(151)
Transmembrane Domain 5:
LVVFFLAMLVLMAVLYVHML(152)
Transmembrane Domain 6:
VTLTILLGIFFLCWGPFFLHLTLIVL
(153)
Transmembrane Domain 7:
FNLFLALIICNAIIDPLIYAF(154)

HUMAN ANGIOTENSIN AT2 TYPE 1 RECEPTOR
Transmembrane Domain 1:
FVMIPTLYSIIFVVGIFGNSLVVIV
(Sequence ID NO:155)
Transmembrane Domain 2:
VFLLNLALADLCFLLTLPLWAVY
(Sequence ID NO:156)
Transmembrane Domain 3:
IASASVSFNLYASVFLLTCLSI
(Sequence ID NO:157)
Transmembrane Domain 4:
TMLVAKVTCIIIWLLAGLASLP
(Sequence ID NO:158)
Transmembrane Domain 5:
PIGLGLTKNILGFLFPFLIILTS
(Sequence ID NO:159)
Transmembrane Domain 6:
IIMAIVLFFFFSWIPHQIFTF
(Sequence ID NO:160)
Transmembrane Domain 7:
IADIVDTAMPITICIAYFNNCL
(Sequence ID NO:161)

TABLE 4B

HUMAN NEUROPEPTIDE NPY5 RECEPTOR

Transmembrane Domain 1:
QYFLIGLYTFVSLLGFMGNLLIL
(Sequence ID NO:162)
Transmembrane Domain 2:
TVNFLIGNLAFSDILVVLFCS
(Sequence ID NO:163)
Transmembrane Domain 3:
IMPFLQCVSVLVSTLILISIAI
(Sequence ID NO:164)
Transmembrane Domain 4:
FLIATVWTLGFAICSPLPVFHS
(Sequence ID NO:165)
Transmembrane Domain 5:
FTISLLLVQYILPLVCLTVSHT
(Sequence ID NO:166)
Transmembrane Domain 6:
LTILILVFAVSWMPLHLFHVVT
(Sequence ID NO:167)
Transmembrane Domain 7:
LVYCICHLLGMMSCCLNPILYGFL
(Sequence ID NO:168)

HUMAN MDR1-P Glycoprotein

Transmembrane Domain 1:
VVGTLAAIIHGAGLPLMMLVF
(Sequence ID NO:169)
Transmembrane Domain 2:
GIGAGVLVAAYIQVSFWCLAA
(Sequence ID NO:170)
Transmembrane Domain 3:
IGMFFQSMATFFTGFIVGFT
(Sequence ID NO:171)
Transmembrane Domain 4:
LVILAISPVLGLSAAVWAKII
(Sequence ID NO:172)
Transmembrane Domain 5:
ISIGAAFLLIYASYALAFWYG
(Sequence ID NO:173)
Transmembrane Domain 6:
YSIGOVLTVFFSVLIGAFSVG
(Sequence ID NO:174)
Transmembrane Domain 7:
FVVGVFCAIINGGLQPAFAII
(Sequence ID NO:175)
Transmembrane Domain 8:
LLFLALGIISFITFFLQGFTF
(Sequence ID NO:176)
Transmembrane Domain 9:
LAVITQNIANLGTGIIISFIY
(Sequence ID NO:177)
Transmembrane Domain 10:
GWQLTLLLLAIVPIIAIAGVV
(Sequence ID NO:178)
Transmembrane Domain 11:
IFGITFSFTQAMMYFSYAGCF
(Sequence ID NO:179)
Transmembrane Domain 12:
LLVFSAVVFGAMAVGQVSSF
(Sequence ID NO:180)

We claim:

1. An antagonist for inhibiting the function of a prokaryotic or eukaryotic integral membrane protein having at least one transmembrane domain, the antagonist comprising a peptide having an amino acid sequence of at least four consecutive amino acids selected from the amino acid sequence of said at least one transmembrane domain.

2. The antagonist of claim 1 comprising a peptide having an amino acid sequence of at least ten consecutive amino acids selected from the amino acid sequence of said at least one transmembrane domain.

3. The antagonist of claim 1 comprising a peptide having an amino acid sequence of at least fifteen consecutive amino acid selected from the amino acid sequence of said at least one transmembrane domain.

4. The antagonist of claim 1 comprising a peptide having an amino acid sequence of at least twenty consecutive amino acids selected from the amino acid sequence of said at least one transmembrane domain.

5. The antagonist of claim 1 comprising a peptide having the amino acid sequence of said at least one transmembrane domain or an effective fragment or analogue thereof.

6. The antagonist of any of claims 1 to 5 wherein the integral membrane protein is a prokaryotic or eukaryotic plasma membrane protein.

7. The antagonist of any of claims 1 to 5 wherein the

integral membrane protein is a prokaryotic or eukaryotic intracellular membrane.

8. The antagonist of claim 6 wherein the integral membrane protein is a mammalian plasma membrane protein.

9. The antagonist of claim 1 wherein the integral membrane protein has a plurality of transmembrane domains and wherein the peptide comprises the amino acid sequence of any one of said plurality of transmembrane domains or a fragment or analogue thereof.

10. The antagonist of any of claims 1 to 9 wherein the integral membrane protein is a human protein.

11. The antagonist of claim 8 wherein the integral membrane protein is a protein selected from the group consisting of

(a) a G-protein coupled receptor;
(b) a tyrosine kinase receptor;
(c) an ion channel;
(d) an ion channel receptor;
(e) a channel protein;
(f) an antigen receptor;
(g) a transporter protein; and
(h) an immune receptor.

12. The antagonist of claim 11 wherein the integral membrane protein is a G-protein coupled receptor selected from the group consisting of

(a) a dopamine receptor;
(b) an adrenergic receptor;
(c) an adenosine receptor;
(d) a vasopressin type 2 receptor;

(e) a chemokine receptor;
(f) a serotonin receptor;
(g) an opioid receptor;
(h) an angiotensin receptor;
(i) a neuropeptide receptor; and
(j) a melanocyte stimulating hormone receptor.

13. The antagonist of claim 12 wherein the integral membrane protein is a D1 dopamine receptor and the antagonist is a peptide selected from the group consisting of
(a) ILTACFLSLLILSTLLGNTLVCAAV;
(b) FFVISLAVSDLLVAVLVMPWKAVAEIA;
(c) NIWVAFDIMCSTASILNLCVISVD;
(d) AAFILISVAWTLSVLISFIPVQLSW;
(e) TYAISSSVISFYIPVAIMIVTYTRI;
(f) TLSVIMGVFVCCWLPFFILNCILPFC;
(g) FDSNTFDVFVWFGWANSSLNPIIYAFNAD and
(h) an effective analogue or fragment of (a) to (g).

14. The antagonist of claim 12 wherein the integral membrane protein is a D2 dopamine receptor and the antagonist is a peptide selected from the group consisting of
(a) ATLLTLLIAVIVFGNVLVCMAVS;
(b) LIVSLAVADLLVATLMPWVVYLEVV;
(c) IVFTLDVMMCTASILNLCAISI;
(d) VTVMISIVWVLSFTISCPLLFGL;
(e) PAFVVYSSIVSFYVPFIVTLLVYI;
(f) MLAIVLGVFIICWLPFFITHILN;
(g) VLYSAFTWLGYVNSAVNPIIYTTF and
(h) an effective analogue or fragment of (a) to (g).

15. The antagonist of claim 12 wherein the integral membrane protein is a D2 dopamine receptor and the antagonist is a peptide selected from the group consisting of

(a) YATLLTLLIAVIVFGNVLVC;
(b) VSLAVADLLVATLVMPWVVY;
(c) TLDVMMCTASILNLCAISID;
(d) RVTVMISIVWVLSFTISCPL;
(e) PAFVVYSSIVSFYVPFIVTL;
(f) LAIVLGVFIICWLPFFITHI;
(g) LYSAFTWLGYVNSAVNPIIY; and
(h) TWLGYVNSA.

16. The antagonist of claim 12 wherein the integral membrane protein is a β1-adrenergic receptor and the antagonist is a peptide selected from the group consisting of

(a) GMGLLMALIVLLIVAGNVLVIVAI;
(b) IMSLASADLVMGLLVVPFGATIVV
(c) ELWTSVDVLCVTASIETLCFIALD
(d) RGLVCTVWAISALVSFLPILMHWW
(e) RAYAIASSVVSFYVPLCIMAFVYL
(f) LGIIMGVFTLCWLPFFLANVVKAF
(g) RLFVFFNWLGYANSAFNPIIYCRS; and
(h) an effective analogue or fragment of (a) to (g).

17. The antagonist of claim 12 wherein the receptor is a β1-adrenergic receptor and the antagonist is the peptide FFNWLGYANSAFNP or GYANSAFNP.

18. The antagonist of claim 12 wherein the receptor is an α1A-adrenergic receptor and the antagonist is a peptide selected from the group consisting of

(a) GVGVGFLAAFILMAVAGNLLVILSV;
(b) FIVNLAVADLLLSATVLPFSATMEVL;
(c) DVWAAVDVLCCTASILSLCTISV;
(d) AAILALLWVVALVVSVGPLLGWKEP;
(e) AGYAVFSSVCSFYLPMAVIVVMYC;
(f) LAIVVGVFVLCWFPFFFVLPLGSL;
(g) EGVFKVIFWLGYFNSCVNPLIYPCS; and
(h) an effective analogue or fragment of (a) to (g).

19. The antagonist of claim 12 wherein the receptor is an α1A-adrenergic receptor and the antagonist is the peptide VFKVIFWLGYFNSCVN or VFKVIFWLGYFNS.

20. The antagonist of claim 12 wherein the receptor is a vasopressin type 2 receptor and the antagonist is a peptide selected from the group consisting of

(a) AELALLSIVFVAVALSNGLVLAALA;
(b) IGHLCLADLAVALFQVLPQLAW;
(c) AVKYLQMVGMYASSYMILAMTL;
(d) VLVAWAFSLLLSLPQLFIFAQ;
(e) TYVTWIALMVFVAPTLGIA;
(f) MTLVIVVVYVLCWAPFFLVQLW; and
(g) LLMLLASLNSCTNPWIYASF.

21. The antagonist of claim 12 wherein the receptor is vasopressin type 2 receptor and the antagonist is the peptide LMLLASLNSCTNPWIY.

22. The antagonist of claim 12 wherein the integral membrane protein is a CCR5 receptor and the antagonist is

a peptide selected from the group consisting of

(a) LLPPLYSLVFIFGFVGNMLVILILINC;

(b) LLNLAISDLFFLLTVPFWAHY;

(c) LLTGLYFIGFFSGIFFIILLTI;

(d) VTFGVVTSVITWVVAVFASLPGIIF;

(e) VILGLVLPLLVMVICYSGIL;

(f) LIFTIMIVYFLFWAPYNIVLLLNTF;

(g) AMQVTETLGMTHCCINPIIYAFVG; and

(h) an effective analogue or fragment of (a) to (g).

23. The antagonist of claim 12 wherein the receptor is CCR5 receptor and the antagonist is the peptide LYSLVFIFGFVGN or MQVTETLGMT.

24. The antagonist of claim 11 wherein the integral membrane protein is a tyrosine kinase receptor selected from the group consisting of

(a) an epidermal growth factor receptor;

(b) a fibroblast growth factor receptor;

(c) a vascular endothelial growth factor receptor; and

(d) a tyrosine kinase A receptor.

25. The antagonist of claim 11 wherein the integral membrane protein is epidermal growth factor receptor erb3 and the antagonist is the peptide MALTVIAGLVVIFMMLGGTFL or LTVIAGLVVIF.

26. The antagonist of claim 6 wherein the integral membrane protein is a bacterial energy-dependent transporter.

27. The antagonist of claim 26 wherein the transporter is the E.coli F_1F_0 ATPase and the antagonist is a peptide

selected from the group consisting of

(a) MAAAVMMGLAAIGAAIGIGILGG;

(b) LAAIGAAIGIGIL;

(c) NATILGQAIAFVLFVLFCM;

(d) GQAIAFVLFVL; and

(e) an effective analogue or fragment of (a) to (d).

28. The antagonist of claim 8 wherein the integral membrane protein is a mammalian energy-dependent transporter.

29. The antagonist of claim 8 wherein the integral membrane protein is a transporter protein.

30. The antagonist of claim 29 wherein the integral membrane protein is a dopamine transporter protein and the antagonist is a peptide selected from the group consisting of

(a) FLLSVIGFAVDLANVWRFPYL;

(b) GAFLVPYLLMVIAGMPLFYM;

(c) GVGFTVILISLYVGFFYNVII;

(d) WQLTACLVLVIVLLYFSLW;

(e) VVWITZTMPYVVLTALLL;

(f) VCFSLGVGFGVLIAFSSY;

(g) IVTTSINSLTSFSSGFVVFSFL;

(h) LPLSSAWAVVFFIMLLTGLI;

(i) LFTLFIVLATFLLSLFCVT;

(j) GTSILFGVLIEAIGVAWFYGV;

(k) LCWKLVSPCFLLFVVVVSIV;

(l) LGWVIATSSMAMVPIYAAY; and

(m) an effective analogue or fragment of (a) to (l).

31. The antagonist of claim 29 wherein the integral membrane protein is a dopamine transporter and the

antagonist is the peptide ALGWIIATS or PDWANALGWVIIATS.

32. The antagonist of claim 8 wherein the integral membrane protein is an immune receptor selected from the group consisting of

(a) an immunoglobulin receptor;
(b) a tumour necrosis factor receptor;
(c) a c type lectin;
(d) a cytokine receptor;
(e) an EGF TMY receptor;
(f) a TMR receptor; and
(g) an effective analogue or fragment of (a) to (f).

33. The antagonist of claim 32 wherein the immune receptor is a CD4 receptor and the antagonist is the peptide LIVLGGVAGLLLF or MALIVLGGVAGILLFIGLGIFF.

34. The antagonist of claim 8 wherein the integral membrane protein is an antigen receptor.

35. The antagonist of claim 34 wherein the receptor is a human T cell antigen receptor and the antagonist is the peptide DTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS or an effective analogue or fragment thereof.

36. A method of inhibiting the function of a prokaryotic or eukaryotic integral membrane protein having at least one transmembrane domain, said method comprising contacting an integral membrane protein with a peptide comprising the amino acid sequence of said at least one transmembrane domain or an effective fragment or analogue of said peptide.

37. The method of claim 36 wherein the integral membrane protein is a prokaryotic or eukaryotic plasma membrane protein.

38. The method of claim 36 wherein the integral membrane protein is a prokaryotic or eukaryotic intracellular membrane.

39. The method of claim 37 wherein the integral membrane protein is a mammalian plasma membrane protein.

40. The method of claim 39 wherein the integral membrane protein is contacted with the antagonist of any of claims 1 to 35.

41. A method of preventing or treating a disorder in a mammal characterised by disordered function of an integral membrane protein having at least one transmembrane domain, said method comprising administering to the mammal an effective amount of a peptide comprising the amino acid sequence of said at least one transmembrane domain or an effective fragment or analogue of said peptide.

42. The method of claim 41 wherein the integral membrane protein is a prokaryotic or eukaryotic plasma membrane protein.

43. The method of claim 41 wherein the integral membrane protein is a prokaryotic or eukaryotic intracellular membrane.

44. The method of claim 42 wherein the integral membrane protein is a mammalian plasma membrane protein.

45. The method of claim 44 wherein the peptide administered to the mammal is the antagonist of any of claims 1 to 35.

46. A method of preventing or treating a disorder in a mammal characterised by disordered function of an integral membrane protein having at least one transmembrane domain, said method comprising inserting into cells of the mammal, *in vivo* or *ex vivo*, a nucleotide sequence encoding the antagonist of any of claims 1 to 35.

47. The method of claim 46 wherein the nucleotide sequence is operably coupled to a promoter which will direct expression of the nucleotide sequence in selected target cells of the subject.

48. A transgenic animal wherein a genome of said animal, or of an ancestor thereof, has been modified by at least one recombinant construct and wherein said recombinant construct has introduced a modification selected from the group consisting of insertion of a nucleotide sequence encoding the antagonist of any of claims 1 to 35.

49. A pharmaceutical composition for treatment of hypertension comprising the antagonist of claim 18 or 19 and a pharmaceutically acceptable carrier.

50. A pharmaceutical composition for the treatment of schizophrenia, psychotic disorders, Huntington's Disease and Tourette's syndrome comprising the antagonist of any of claims 14 to 15 and a pharmaceutically acceptable carrier.

51. A pharmaceutical composition for treatment of drug abuse comprising the antagonist of claim 13 and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition for treatment of hypertension, post-myocardial infarction and tachyarrthymias comprising the antagonist of any of claims 16 to 17 and a pharmaceutically acceptable carrier.

53. A diuretic pharmaceutical composition comprising the antagonist of claims 20 or 21 and a pharmaceutically acceptable carrier.

54. A pharmaceutical composition for treatment of HIV infection and AIDS comprising the antagonist of any of claims 22 or 23 and a pharmaceutically acceptable carrier.

55. A pharmaceutical composition for control of cell proliferation in cancer, psoriasis and hyperkeratotic disorders comprising the antagonist of claim 25 and a pharmaceutically acceptable carrier.

56. A pharmaceutical composition for treatment of substance abuse comprising the antagonist of any of claims 30 to 31 and a pharmaceutically acceptable carrier.

57. An antibacterial composition comprising the antagonist of any of claims 26 and 27 and a pharmaceutically acceptable carrier.

58. A method for treating hypertension in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 49.

59. A method for treating schizophrenia, psychotic disorders, Huntington's Disease and Tourette's syndrome comprising administering to the subject an effective amount of the pharmaceutical composition of claim 50.

60. A method of treating drug abuse in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 51.

61. A method of treating hypertension, post-myocardial infarction and tachyarrthymias in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 52.

62. A method of providing diuretic treatment to a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 53.

63. A method of treating HIV infection and AIDS in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 54.

64. A method for controlling cell proliferation in a subject suffering from cancer, psoriasis or a hyperkeratotic disorder comprising administering to the subject an effective amount of the pharmaceutical composition of claim 55.

65. A method of treating substance abuse in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 56.

66. A method of treating a bacterial infection in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 57.

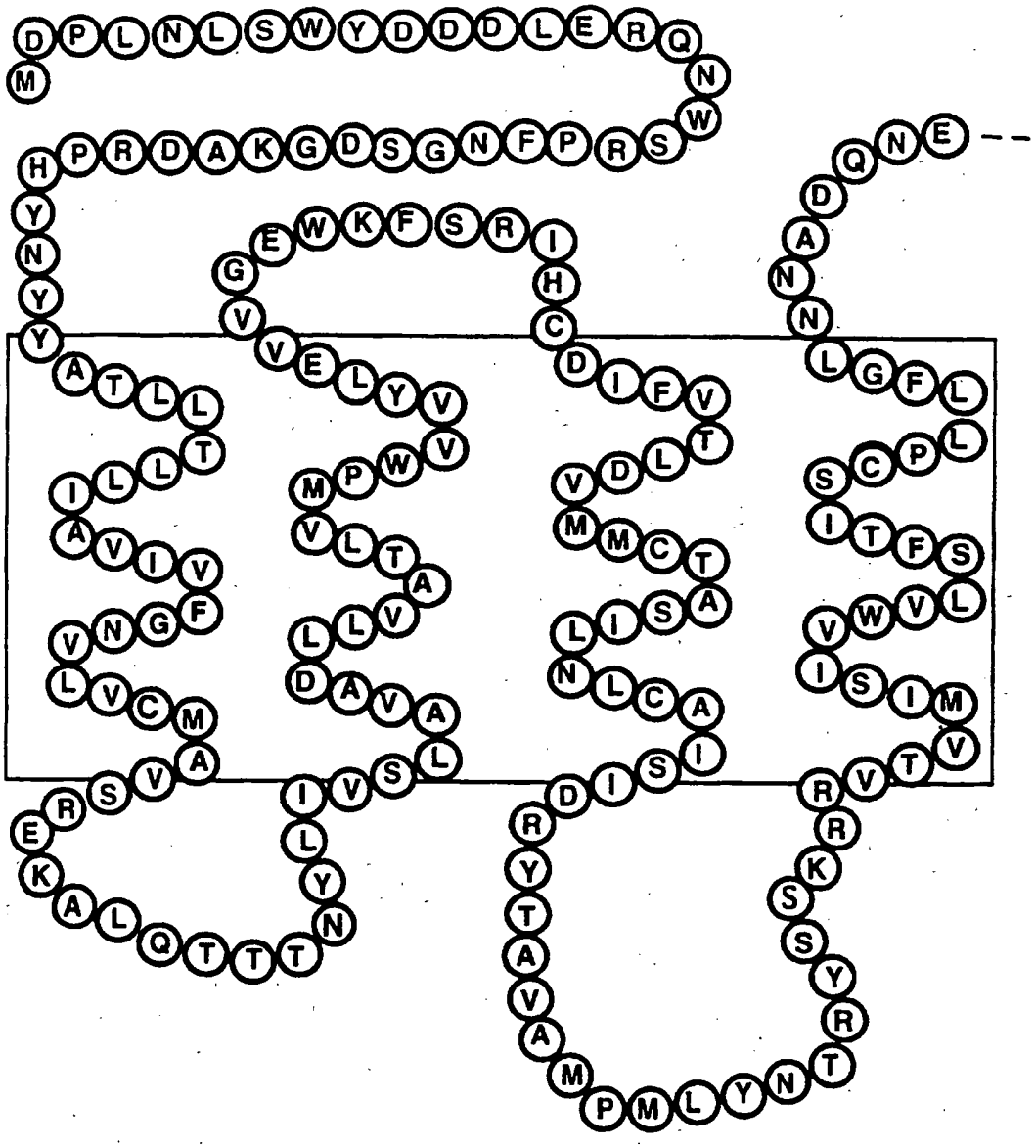


FIG.1A.

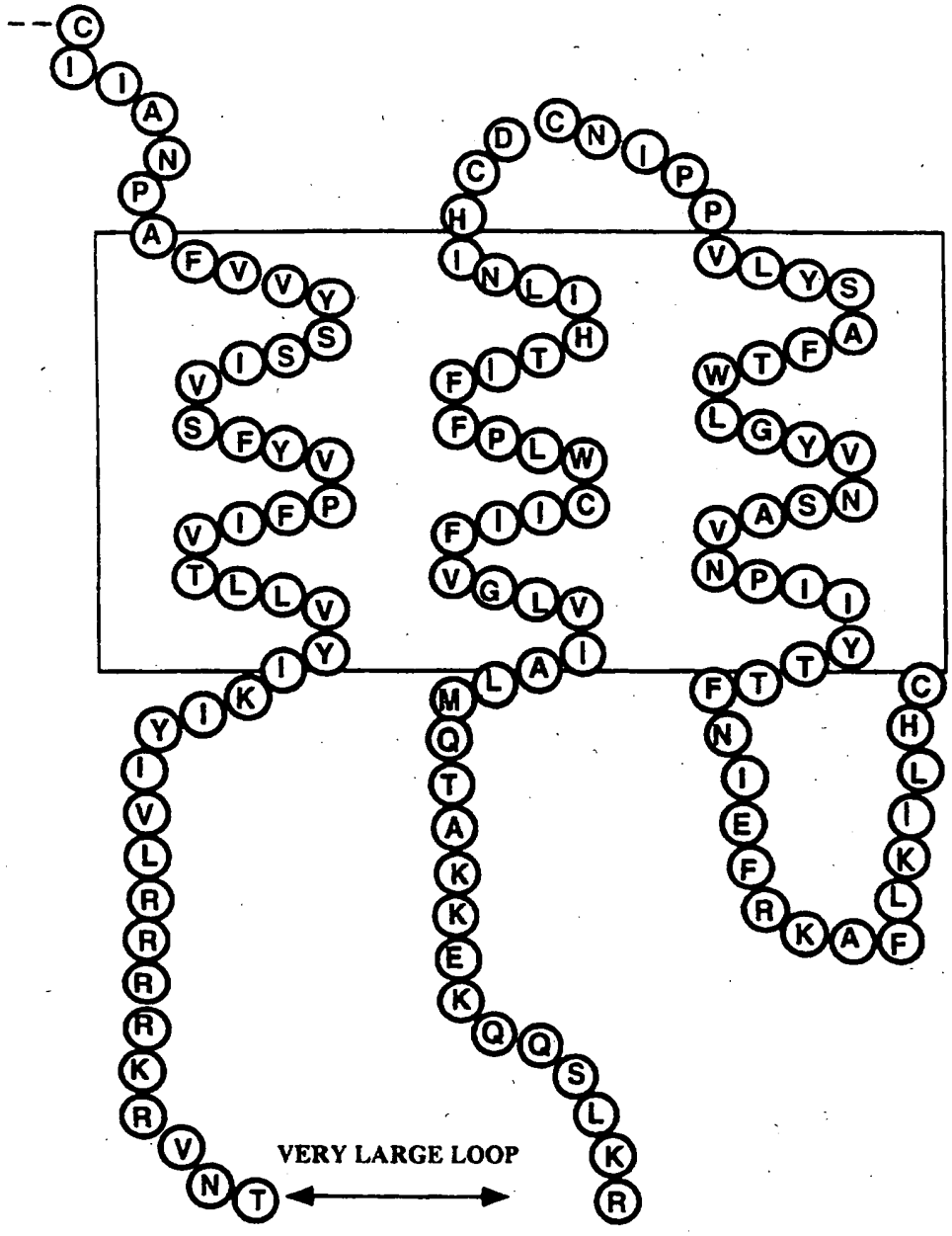


FIG.1B.

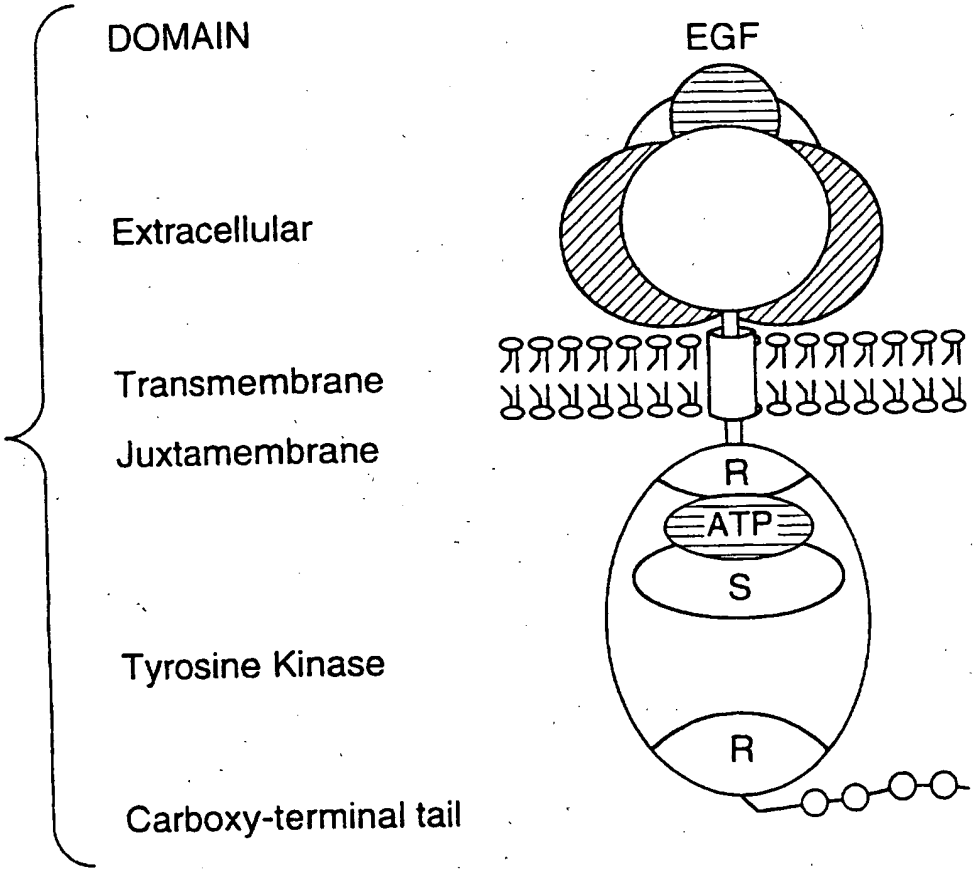

DOMAIN
EGF
Extracellular
Transmembrane
Juxtamembrane
Tyrosine Kinase
Carboxy-terminal tail
R
ATP
S
R

FIG.2

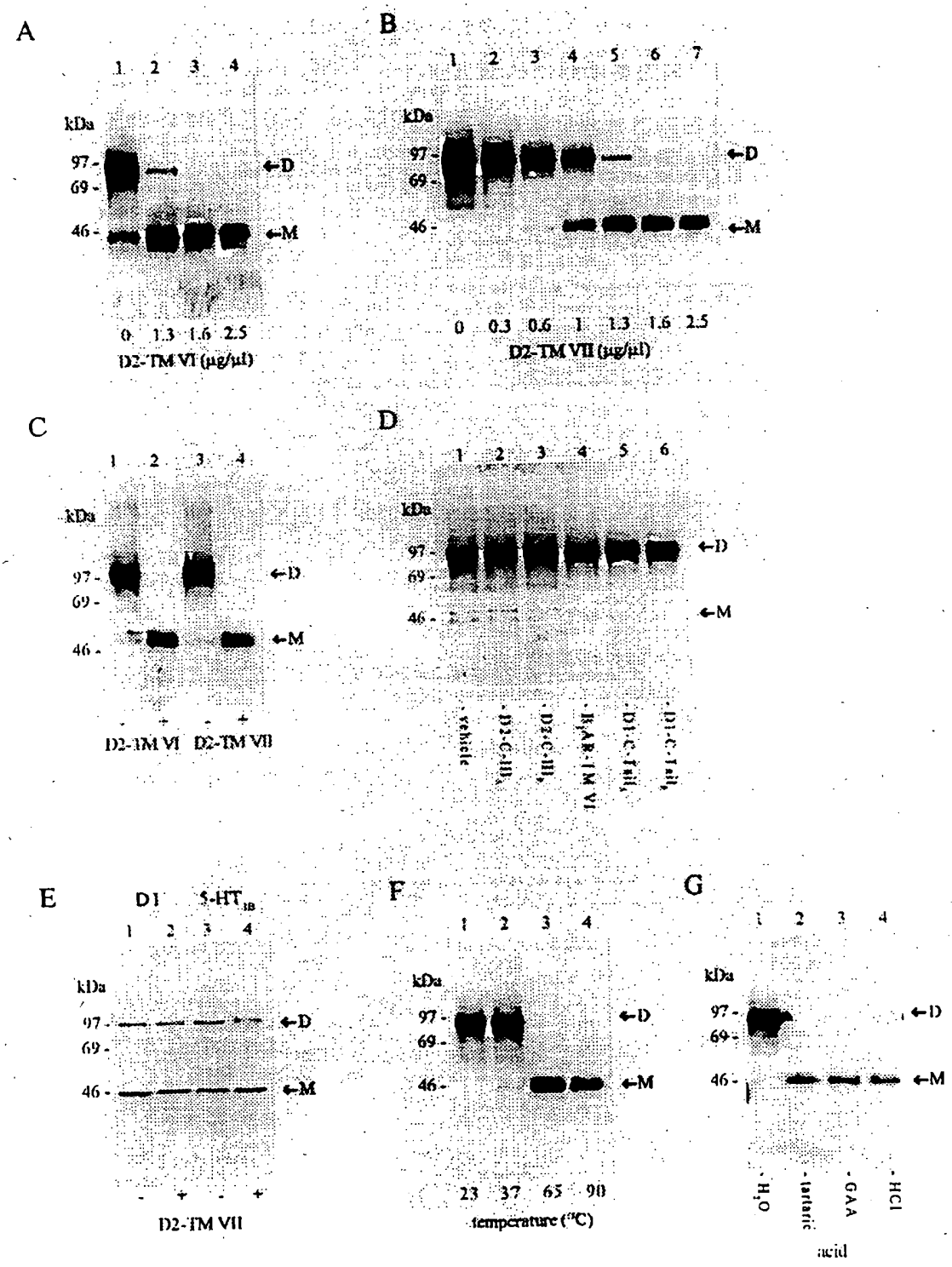

A
1 2 3 4
kDa
97 -
69 -
46 -
←D
←M
0 1.3 1.6 2.5
D2-TM VI (µg/µl)
B
1 2 3 4 5 6 7
kDa
97 -
69 -
46 -
←D
←M
0 0.3 0.6 1 1.3 1.6 2.5
D2-TM VII (µg/µl)
C
1 2 3 4
kDa
97 -
69 -
46 -
←D
←M
- + - +
D2-TM VI D2-TM VII
D
1 2 3 4 5 6
kDa
97 -
69 -
46 -
←D
←M
- vehicle
- D2-C-IIIa
- D2-C-IIIb
- BAR-TM VI
- D1-C-Tail
- D1-C-Tail
E
D1 5-HT1B
1 2 3 4
kDa
97 -
69 -
46 -
←D
←M
- + - +
D2-TM VII
F
1 2 3 4
kDa
97 -
69 -
46 -
←D
←M
23 37 65 90
temperature (°C)
G
1 2 3 4
kDa
97 -
69 -
46 -
←D
←M
- H2O
- tartaric
- GAA
- HCl
acid

FIG. 3.

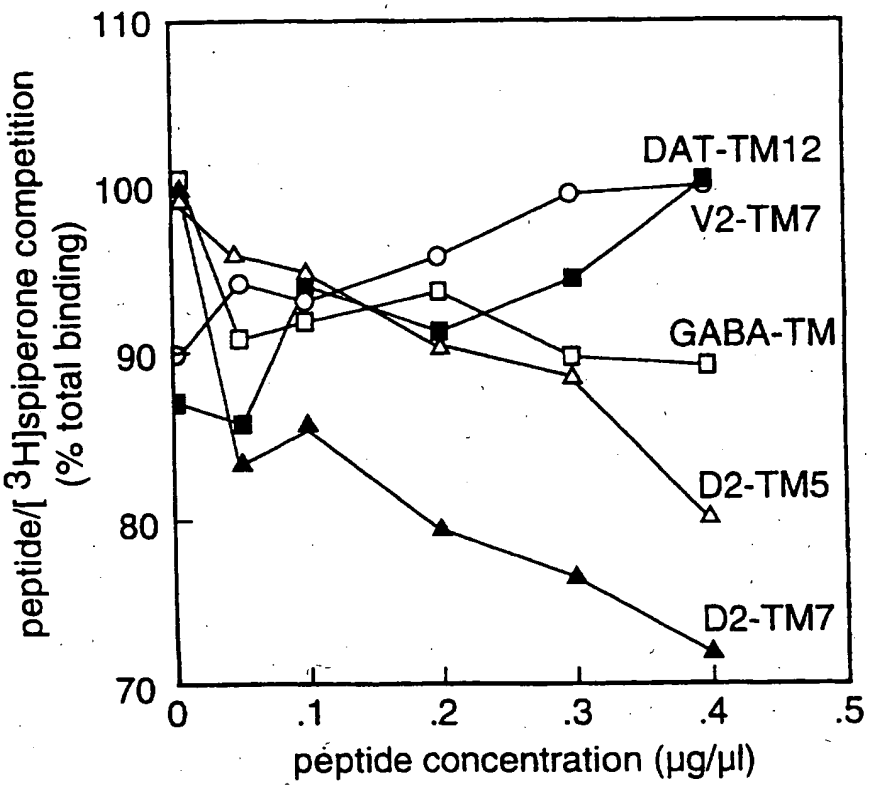

110
100
90
80
70
0
.1
.2
.3
.4
.5
peptide/[3H]spiperone competition
(% total binding)
peptide concentration (µg/µl)
DAT-TM12
V2-TM7
GABA-TM
D2-TM5
D2-TM7

FIG.4

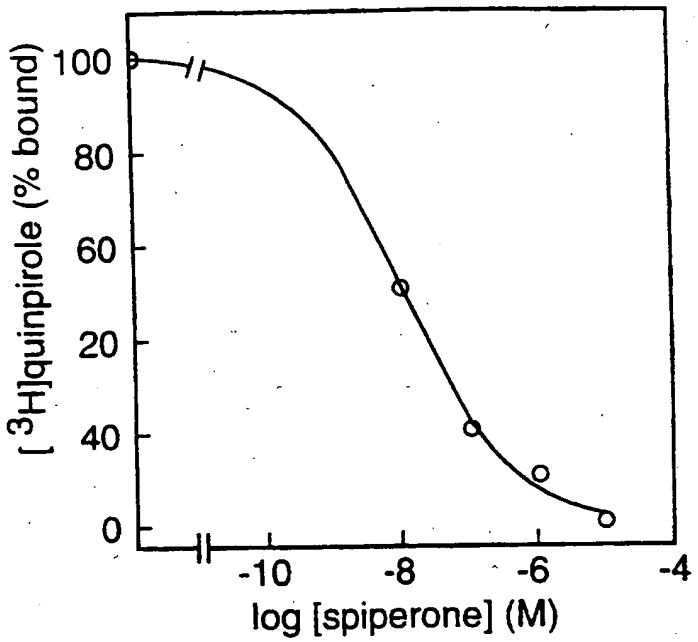

[3H]quinpirole (% bound)
100
80
60
20
40
0
-10
-8
-6
-4
log [spiperone] (M)

FIG.5A

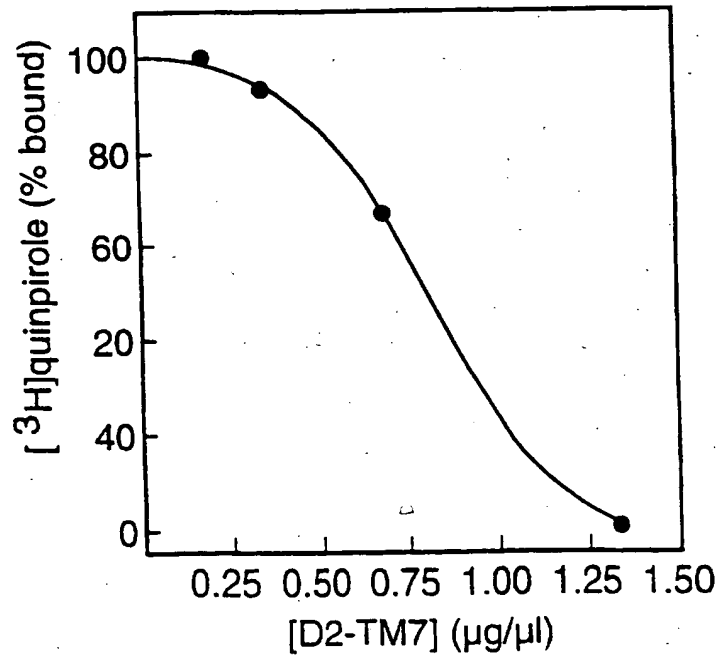

[3H]quinpirole (% bound)
100
80
60
20
40
0
0.25 0.50 0.75 1.00 1.25 1.50
[D2-TM7] (µg/µl)

FIG.5B

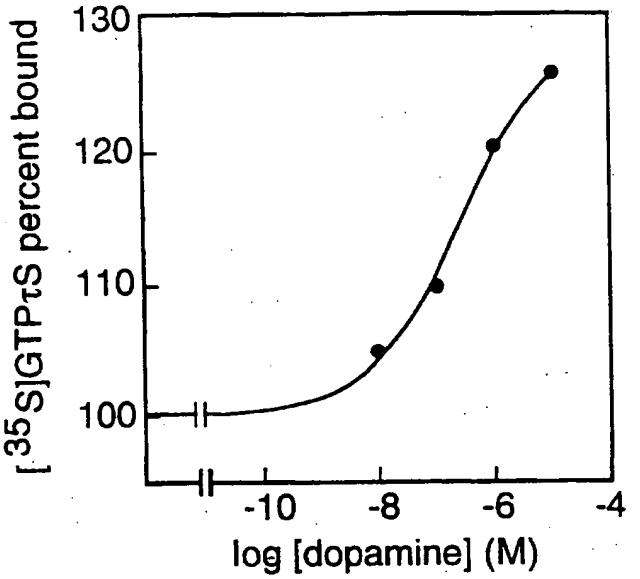

130
120
110
100
[35S]GTPγS percent bound
-10
-8
-6
-4
log [dopamine] (M)

FIG.6A

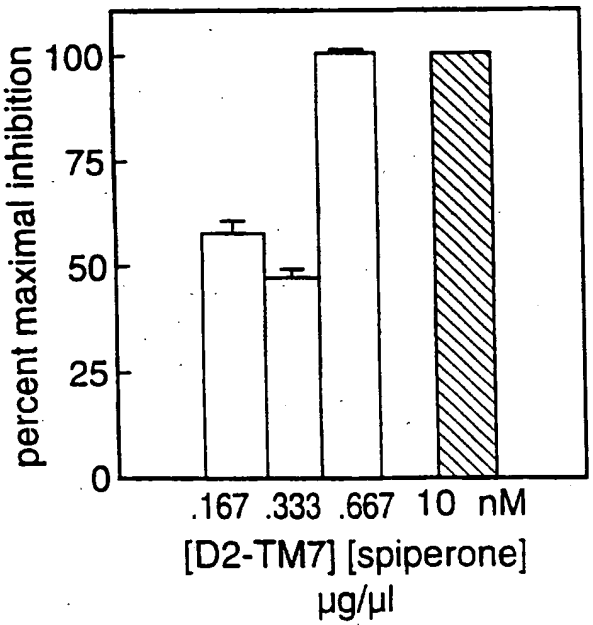

100
75
50
25
0
percent maximal inhibition
.167 .333 .667 10 nM
[D2-TM7] [spiperone]
µg/µl

FIG.6B

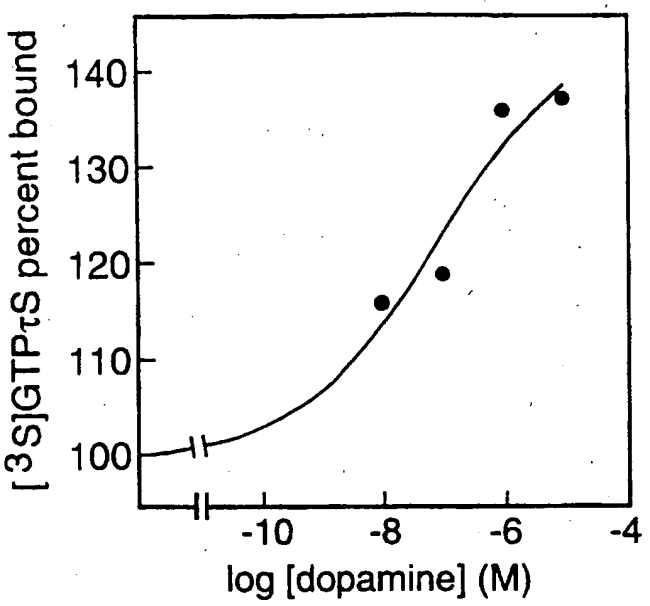

[3S]GTPγS percent bound
140
130
120
110
100
-10
-8
-6
-4
log [dopamine] (M)

FIG.7A

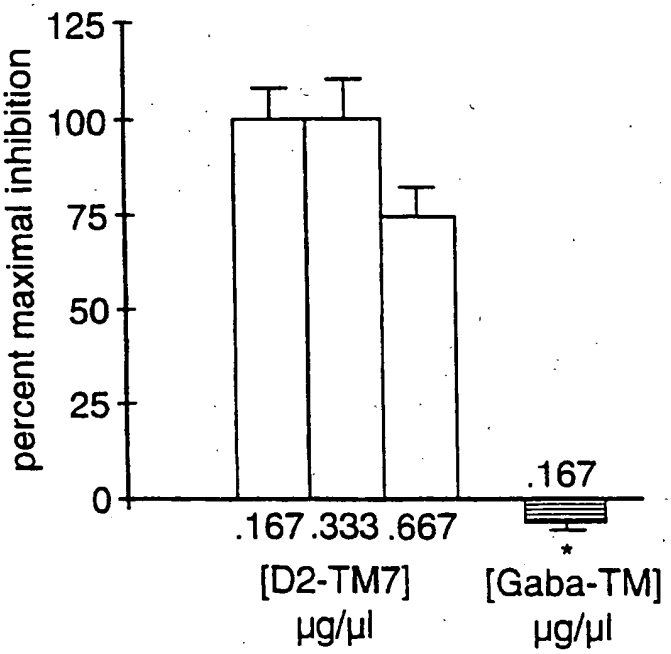

percent maximal inhibition
125
100
75
50
25
0
.167
.167 .333 .667
[D2-TM7]
µg/µl
[Gaba-TM]
µg/µl

FIG.7B

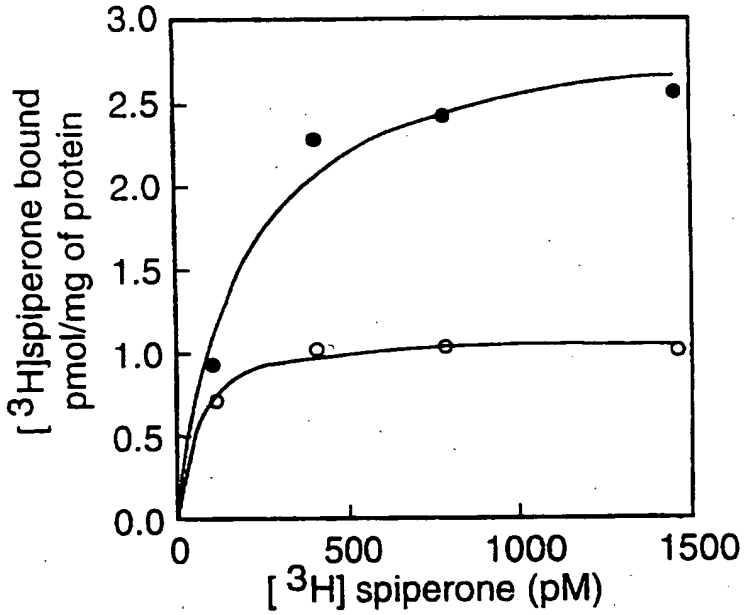

3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
[3H]spiperone bound
pmol/mg of protein
[3H] spiperone (pM)

FIG.8

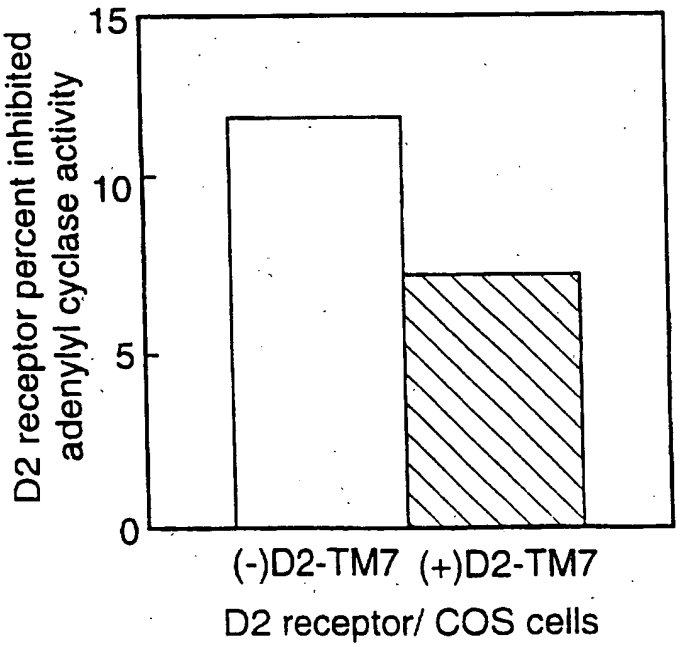

15
10
5
0
D2 receptor percent inhibited
adenylyl cyclase activity
(-)D2-TM7
(+)D2-TM7
D2 receptor/ COS cells

FIG.9

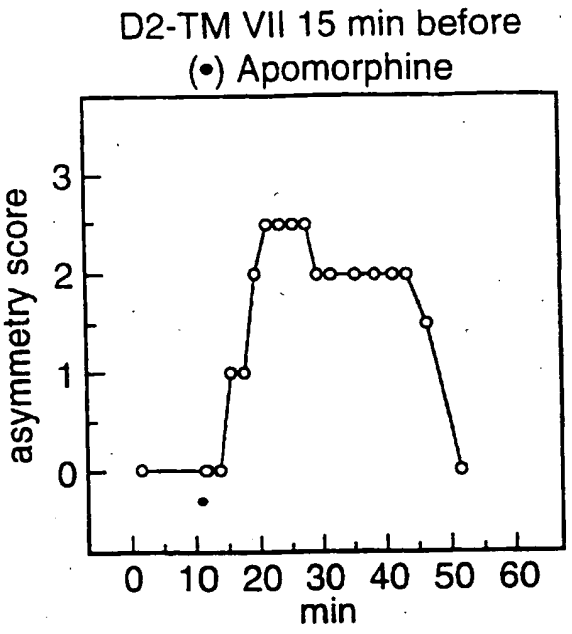


FIG.10A

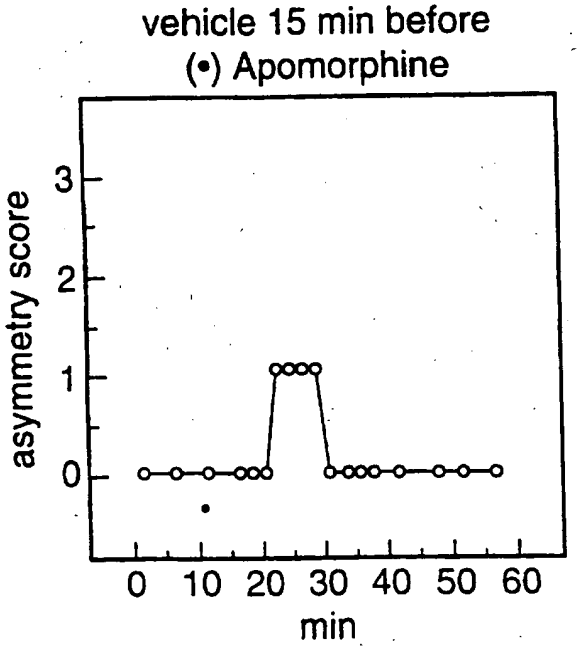


FIG.10B

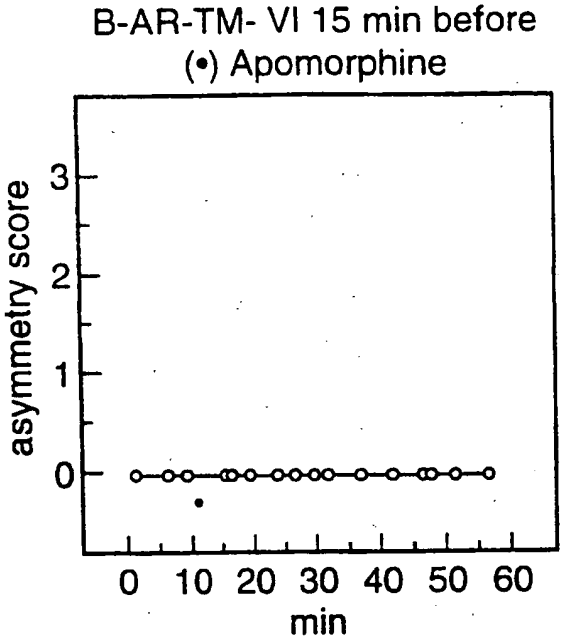


FIG.10C

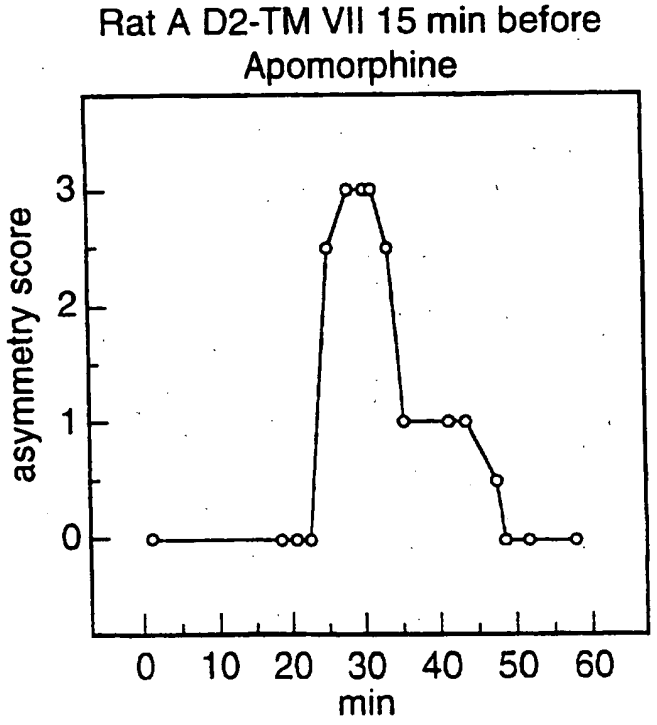


FIG.11

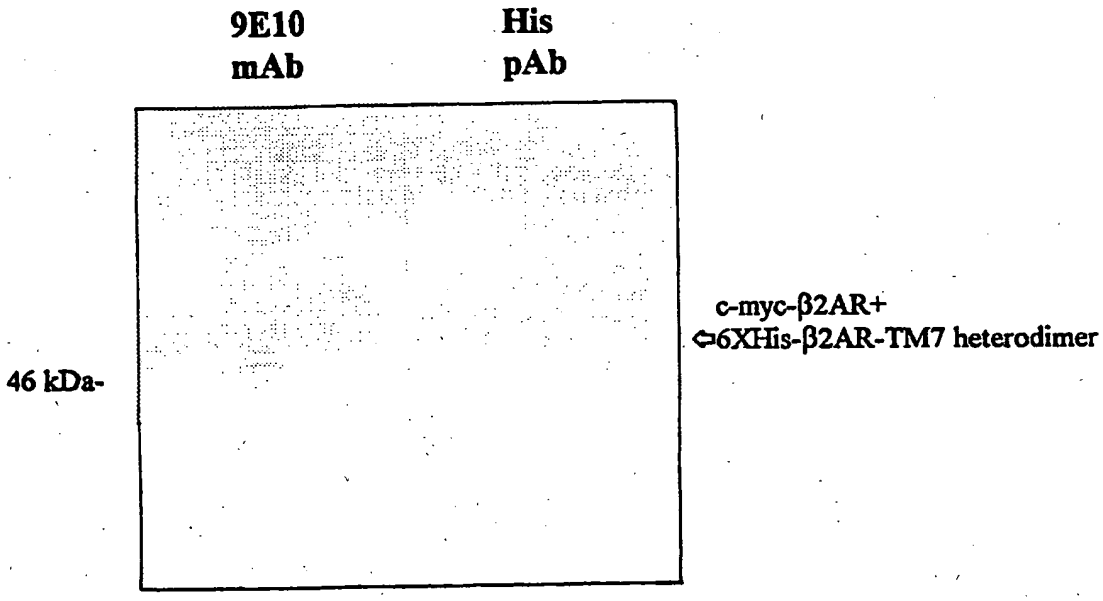


FIG.12.

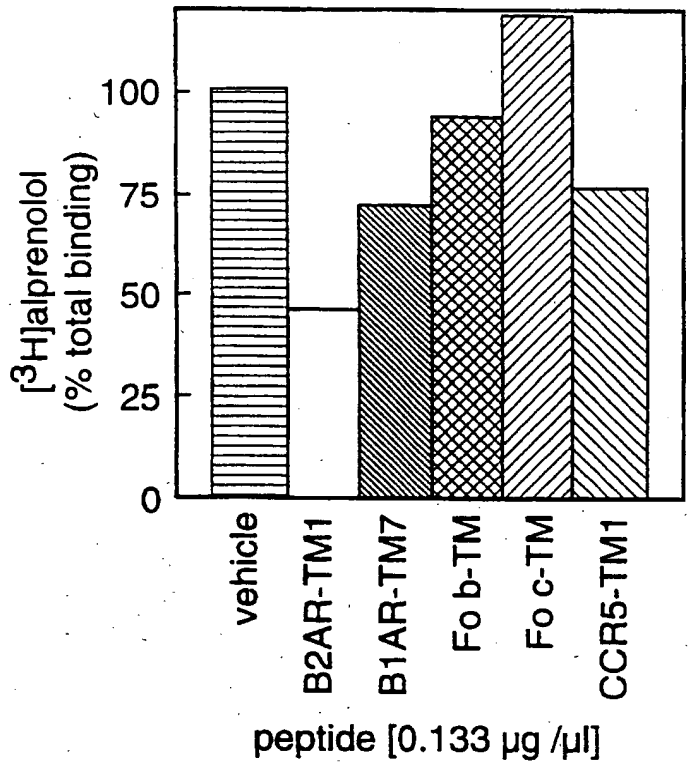


FIG.13A

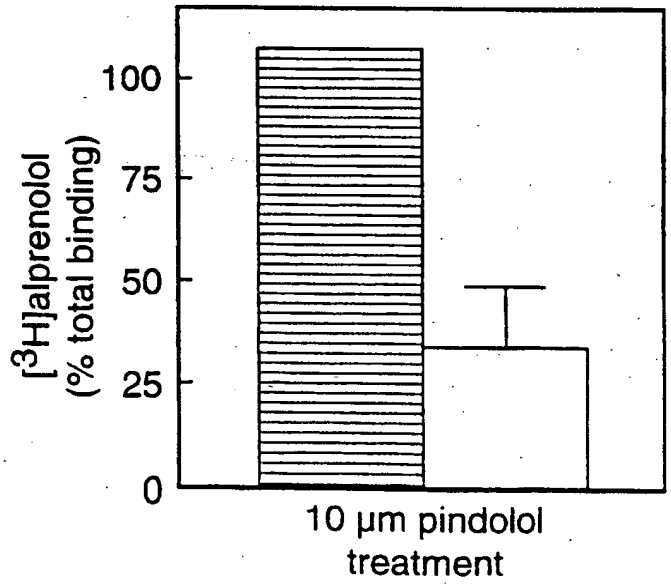


FIG.13B

FIGURE 14A

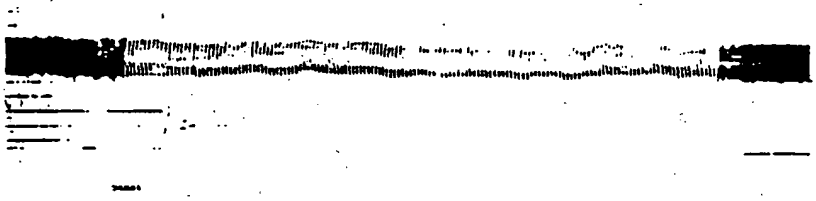


FIGURE 14B

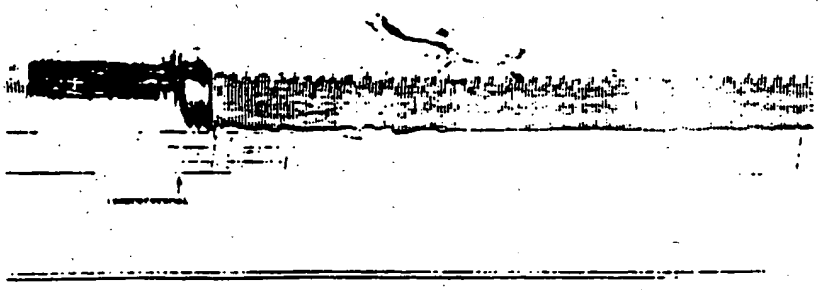


FIGURE 14C

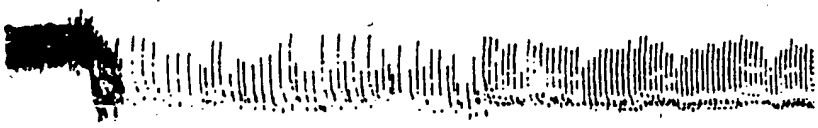


FIGURE 14D

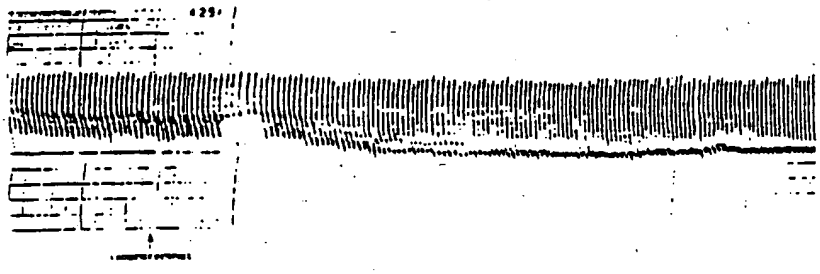


FIGURE 14E

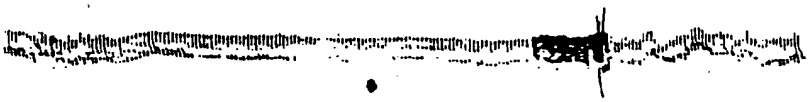


FIGURE 14F

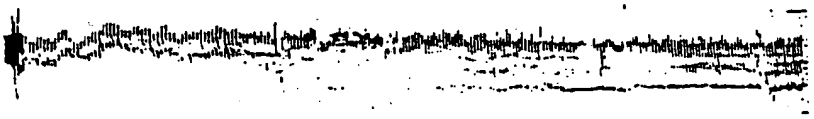


FIGURE 14G

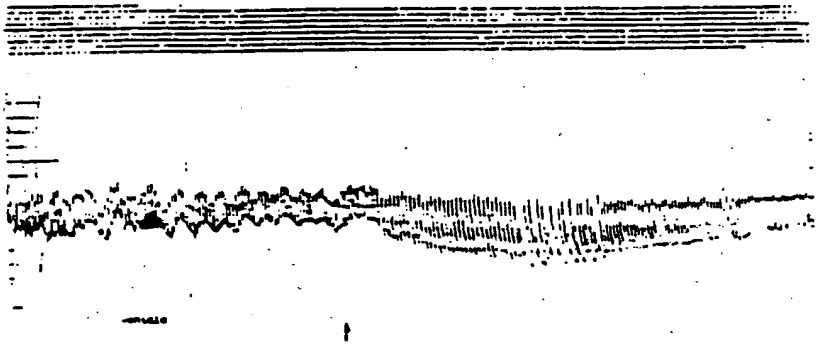


FIGURE 14H

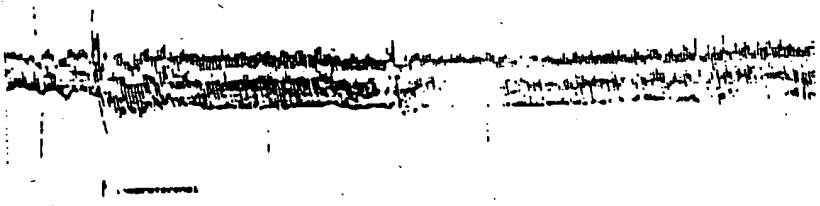


FIGURE 15A

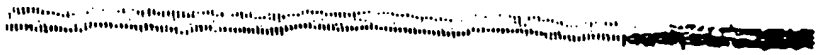


FIGURE 15B

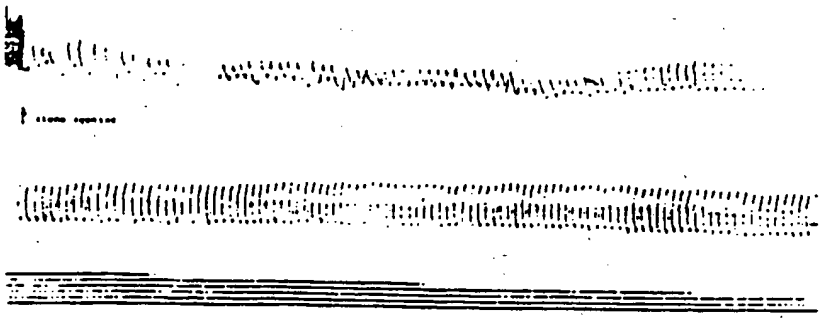


FIGURE 15C

FIGURE 15D

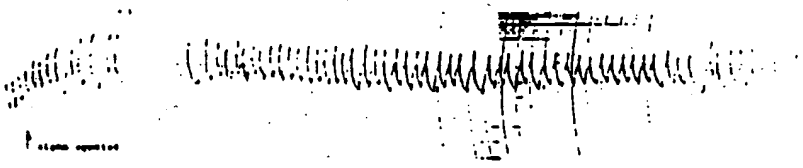


FIGURE 15E

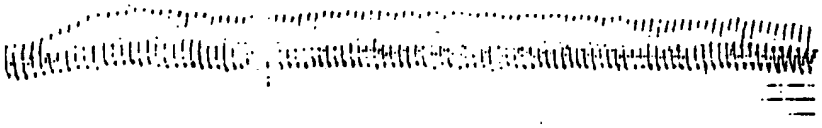


FIGURE 15F

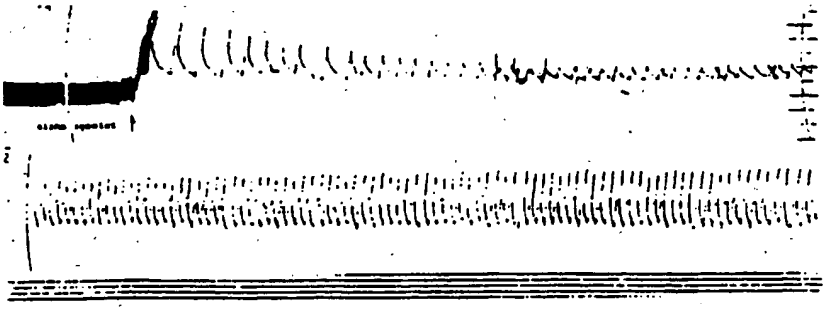


FIGURE 15G

FIGURE 15H

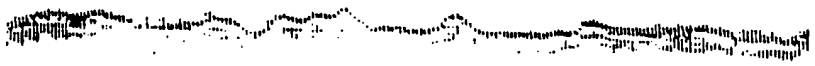


FIGURE 15I

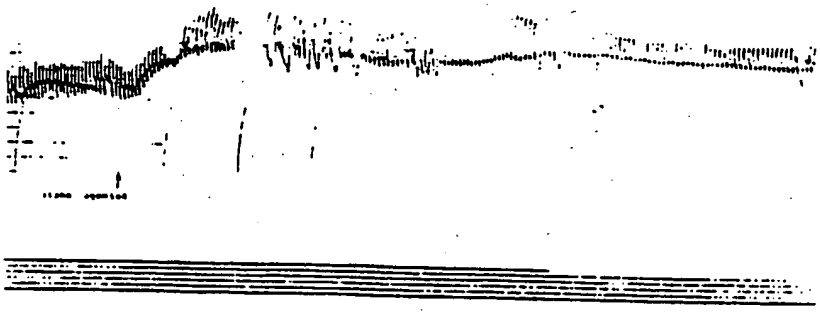


FIGURE 15J

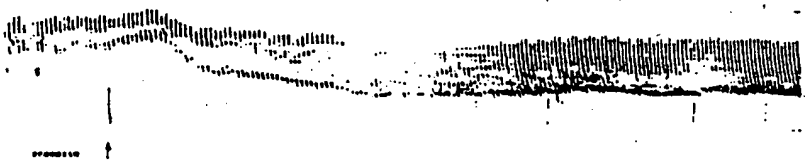
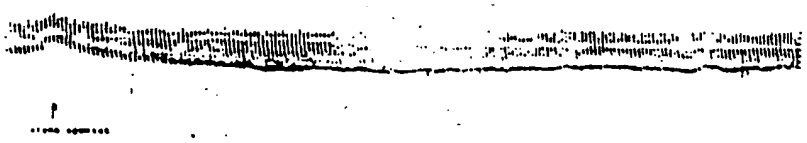


FIGURE 15K



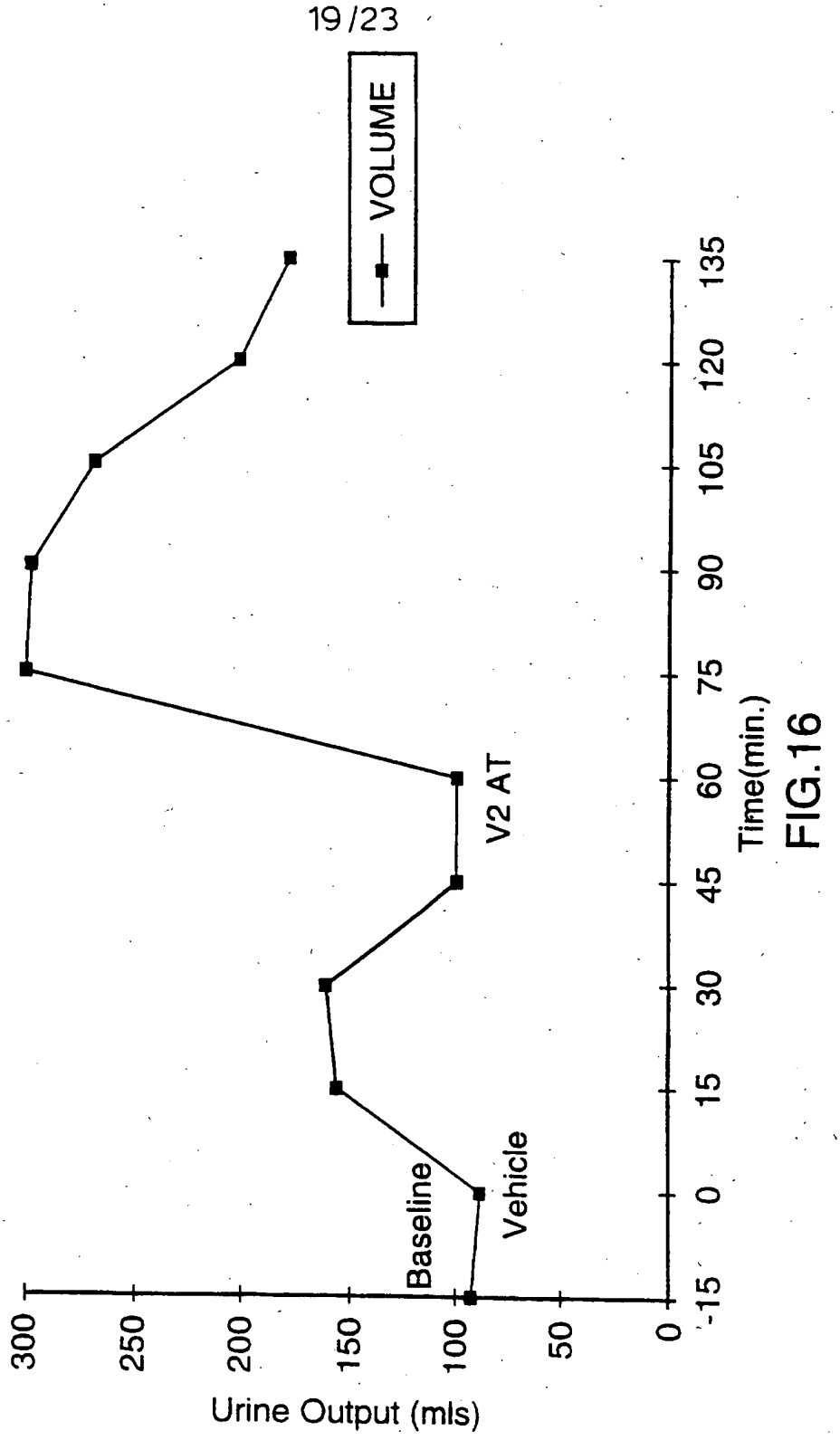

VOLUME
Urine Output (mls)
300
250
200
150
100
50
0
-15
0
15
30
45
60
75
90
105
120
135
Time(min.)
Baseline
Vehicle
V2 AT

FIG.16

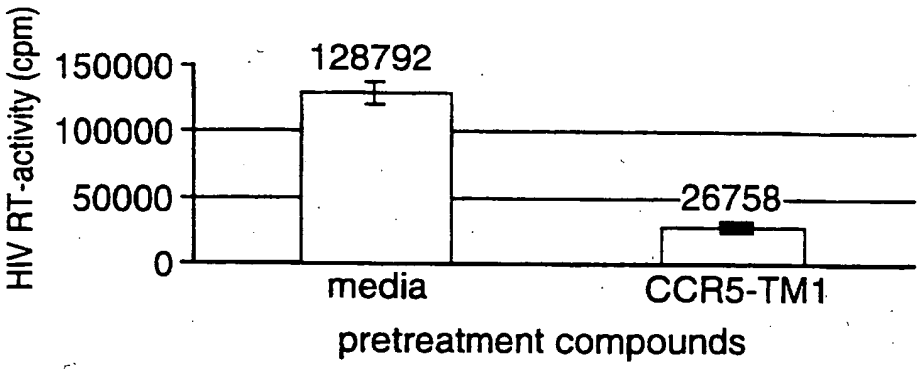


FIG.17A

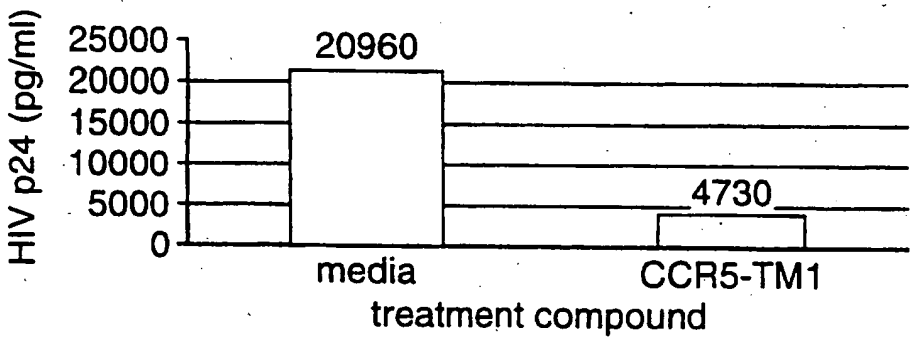


FIG.17B

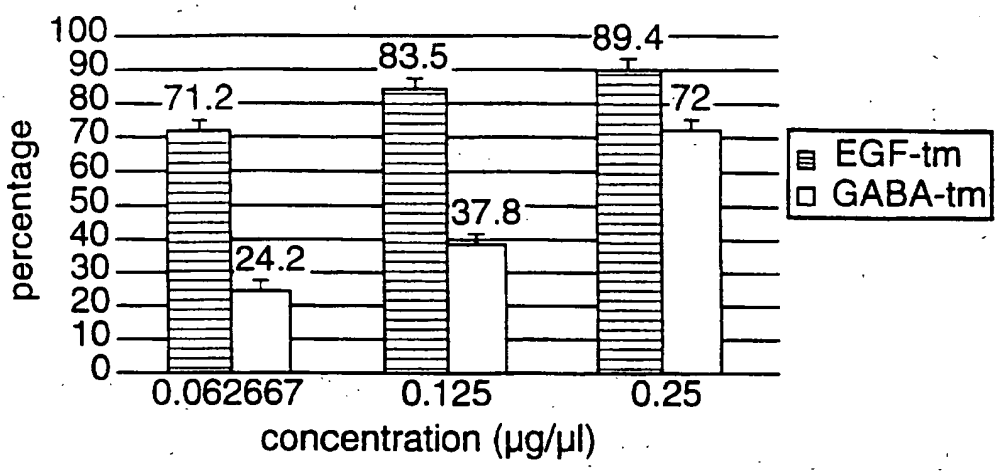

100
90
80
70
60
50
40
30
20
10
0
percentage
71.2
24.2
83.5
37.8
89.4
72
EGF-tm
GABA-tm
0.062667
0.125
0.25
concentration (µg/µl)

FIG.18

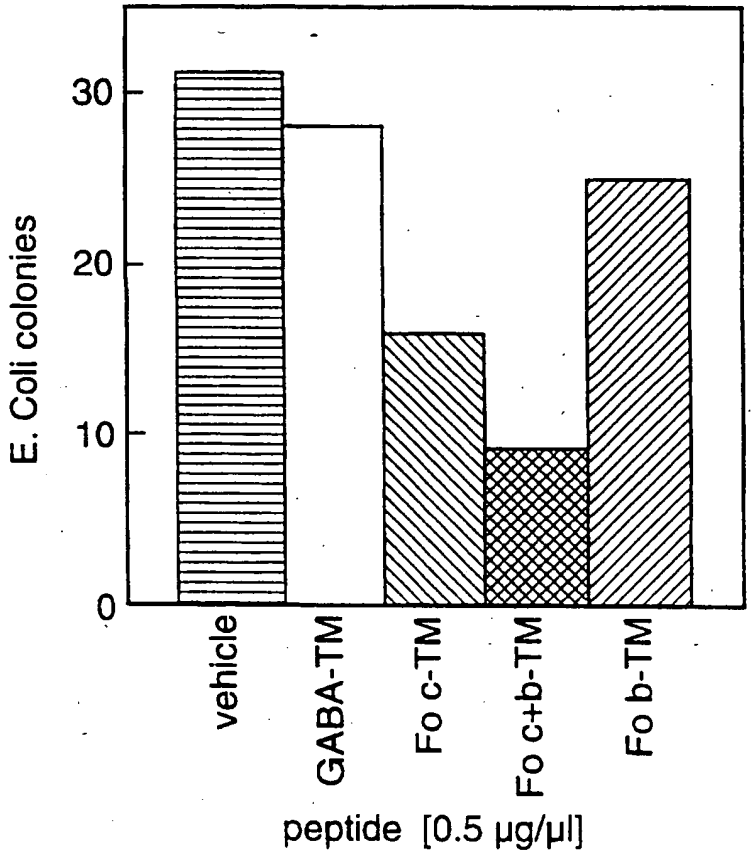

30
20
10
0
E. Coli colonies
vehicle
GABA-TM
Fo c-TM
Fo c+b-TM
Fo b-TM
peptide [0.5 µg/µl]

FIG. 19

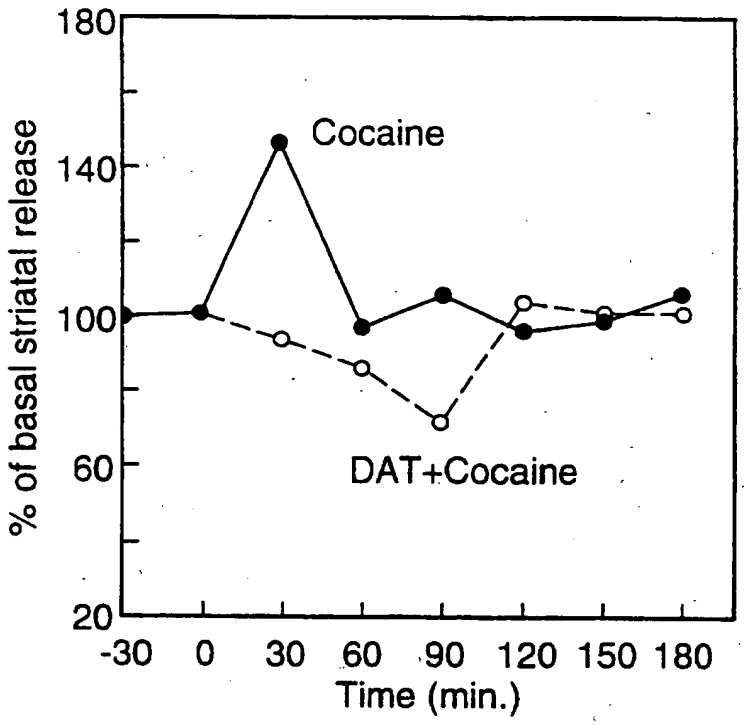

180
140
100
60
20
% of basal striatal release
Cocaine
DAT+Cocaine
-30 0 30 60 90 120 150 180
Time (min.)

FIG.20

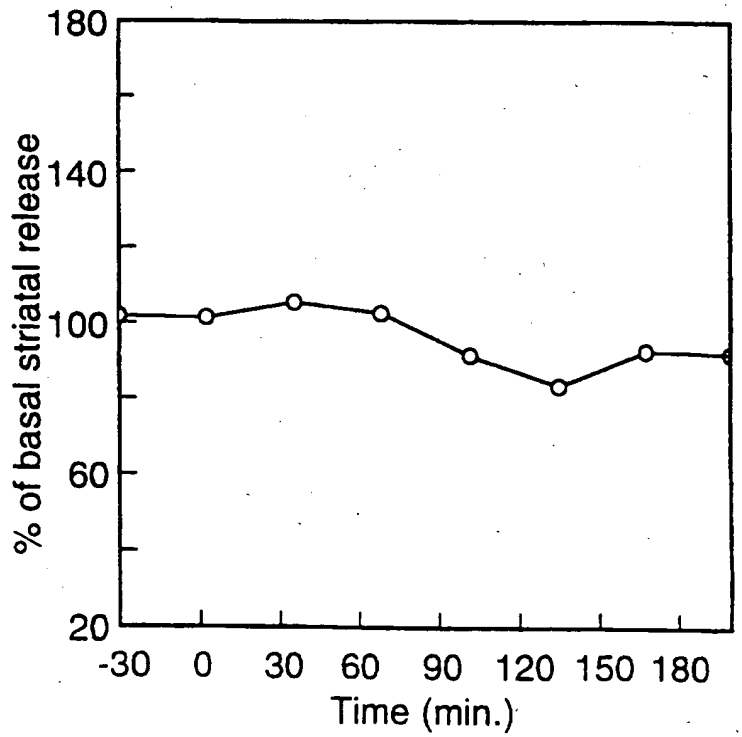

180
140
100
60
20
% of basal striatal release
-30 0 30 60 90 120 150 180
Time (min.)

FIG.21

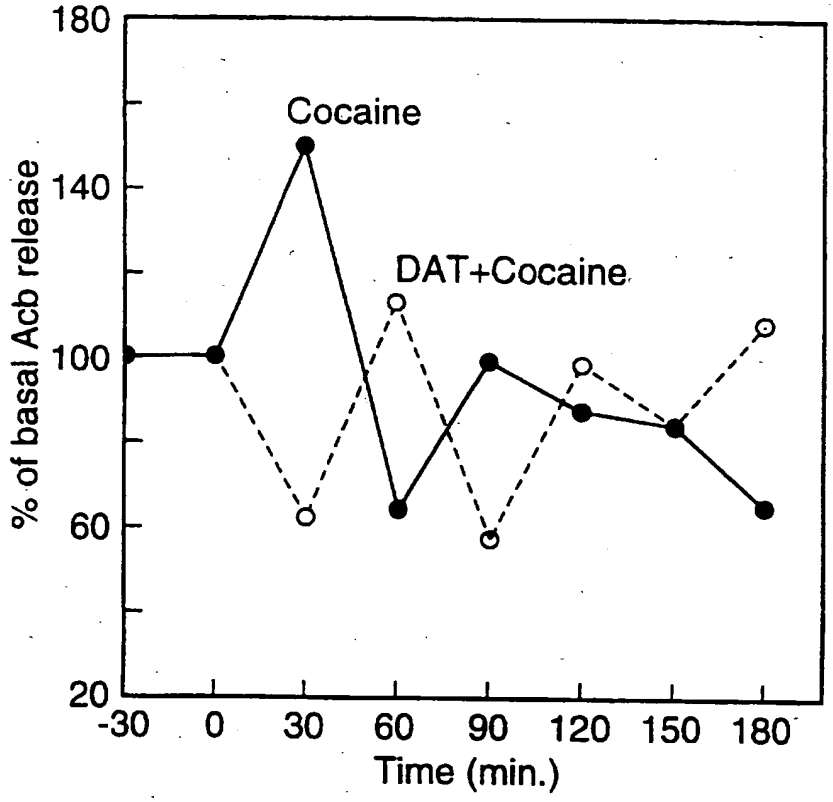

180
140
100
60
20
% of basal Acb release
Cocaine
DAT+Cocaine
-30
0
30
60
90
120
150
180
Time (min.)

FIG.22

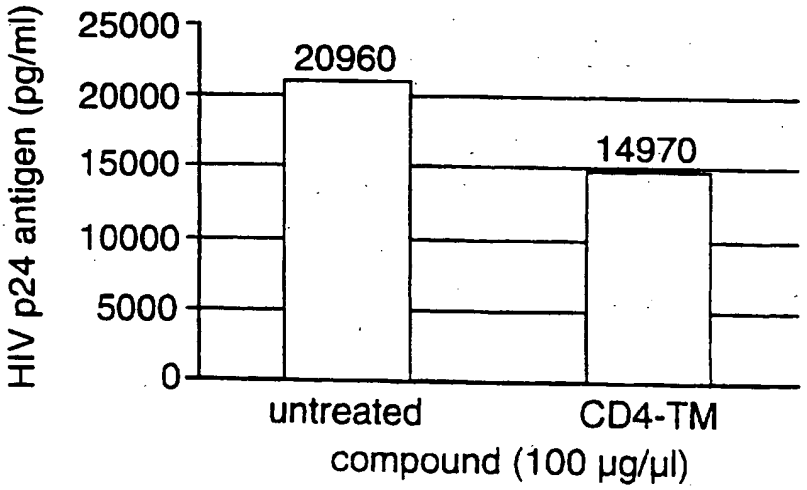

25000
20000
15000
10000
5000
0
HIV p24 antigen (pg/ml)
20960
14970
untreated
CD4-TM
compound (100 µg/µl)

FIG.23